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ENVELHECIMENTO: ASPECTOS MOLECULARES E SUAS IMPLICAÇÕES SOBRE O SISTEMA CARDIOVASCULAR

Arthur José Pontes Oliveira de Almeida

João Pessoa

2017

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Trabalho de Conclusão de Curso apresentado à Coordenação do Curso de Graduação em Farmácia, do Centro de Ciências da Saúde da Universidade Federal da Paraíba, como parte dos requisitos para obtenção do grau de **Bacharel em Farmácia**.

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Aprovado em ___/___/___

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Dedicatória

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Arthur José Pontes Oliveira de Almeida

“Porque as pessoas que são loucas o suficiente para achar que podem mudar o mundo, são aquelas o que o fazem.”

Jack Kerouac

Resumo

RESUMO

ALMEIDA, A.J.P.O.; **Envelhecimento: aspectos moleculares e suas implicações sobre o sistema cardiovascular.** Trabalho de Conclusão de Curso de Farmácia, CCS/UFPB, 2017.

A população mundial acima de 60 anos está crescendo rapidamente, atingindo 22% da população global nas próximas décadas. Apesar do aumento da longevidade global, a saúde das pessoas precisam acompanhar tal crescimento. Várias doenças têm sua prevalência aumentada pela idade, como as doenças cardiovasculares, a principal causa de morbidade e mortalidade no mundo. Compreender os mecanismos biológicos do envelhecimento é fundamental para a busca da saúde cardiovascular. O envelhecimento é caracterizado por um declínio gradual das funções fisiológicas, envolvendo o aumento do número de células senescentes no corpo, várias vias levam à senescência, incluindo o estresse oxidativo e inflamação persistente, além falha da disfunção mitocondrial e autofagia desregulada, levando a uma falha energética, sendo ROS, AMPK, SIRTs, mTOR, IGF-1 e p53 reguladores essenciais do controle metabólico, conectando o envelhecimento aos caminhos que conduzem as doenças. Além disso, a senescência pode ser induzida pela replicação celular, resultante do encurtamento dos telômeros. Tomados em conjunto, é possível desenhar uma via comum unificando o envelhecimento para as doenças cardiovasculares, e o ponto central deste processo, a senescência, pode ser o alvo de novas terapias, levando o tempo de vida saudável levar acompanhar o aumento da longevidade.

Palavras-chave: Envelhecimento; longevidade; vida saudável; senescência; doenças cardiovasculares; dano ao DNA; telômeros; estresse oxidativo; inflamação; disfunção mitocondrial; autofagia; metabolismo.

Abstract

ABSTRACT

ALMEIDA, A.J.P.O.; **Aging: molecular pathways and implications on the cardiovascular system.** Trabalho de Conclusão de Curso de Farmácia. CCS/UFPB, 2017.

The world's population over 60 years is growing rapidly, reaching 22% of the global population in the next decades. Despite the increase in the global longevity, individuals' healthspan needs to follow this growth. Several diseases have its prevalence increased by age, such as cardiovascular diseases, the leading cause of morbidity and mortality in the worldwide. Understanding the aging biology mechanisms is fundamental to the pursuit of cardiovascular health. Aging is characterized by a gradual decline in physiological functions, involving the increased number in the senescent cells into the body. Several pathways lead to senescence, including oxidative stress and persistent inflammation, plus mitochondrial dysfunction and deregulated autophagy, leading to an energy failure, being ROS, AMPK, SIRTs, mTOR, IGF-1 and p53 essential regulators of the metabolic control, connecting aging to the pathways who drive to diseases. In addition, senescence can be induced by cellular replication, resulted from telomere shortening. Taken together, it is possible to draw a common pathway unifying aging to cardiovascular diseases, and the central point of this process, senescence, can be the target for new therapies, which may result in the healthspan matching the lifespan.

Keywords: Aging; lifespan; healthspan; senescence; cardiovascular diseases; DNA damage; telomeres; oxidative stress; inflammation; mitochondrial dysfunction; autophagy; metabolism.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

ADP – Difosfato de adenosina

AMP - 5'-monofosfato de adenosina

AMPK - Proteína quinase ativada por 5’ monofosfato de adenosina

AP-1 – Ativador de proteína 1

AT1 – Receptor para angiotensina II

ATG – Genes relacionados com a autofagia

ATP - Trifosfato de adenosina

BrdU – 5-bromo-2 '-desoxiuridina

CAT – Catalase

CDK - Quinase dependente de ciclina

CMA - Autofagia mediada por chaperonas

COX 2 - Ciclooxigenase -2

cPLA 2 - Fosfolipase A2 citosólica

CR – Restrição calórica

DCV – Doenças cardiovasculares

DDR - Resposta ao dano ao DNA

DDR - Resposta ao dano ao DNA

DNA - Ácido desoxirribonucleico

E2F – Fator E2

ECs - Células endoteliais

eNOS - Sintase de óxido nítrico endotelial

ERKs - Quinases reguladoras de sinal extracelular

FOXO – *Forkhead box O*

GLUT – Transportador de glicose

GPx - Glutationa peroxidase

GR - Glutationa redutase

HIF-1α – Fator induzido por hipóxia 1 alfa

H_2O_2 - Peróxido de hidrogénio

HO• - Radical hidroxil

HO-1 - Hemeoxigenase-1

HOCl - Ácido hipocloroso

HP1 – Proteína heterocromatina 1

ICAM-1 – molécula de adesão intercelular-1

IGF-1 - insulina / fator de crescimento-1

IL– Interleucina

iNOS - Sintase óxido nítrico indutível

iPSC – Células tronco pluripotentes induzidas

JNKs - quinases N-terminais c-jun

LAMP-2A - proteína tipo 2 associada a membrana do lisossomo

MMPs – Metaloproteinases

mtDNA – DNA mitocondrial

mtROS – ROS mitocondrial

mTOR – Alvo mecânico da rapimicina

NAD^+ - Dinucleotídeo de nicotinamida e adenina oxidada

NADH – Dinucleotídeo de nicotinamida e adenina

Nf-kB – Fator nuclear Kappa B

NMN – Mononucleótideo de nicotinamida

NO – Óxido nítrico

Nox - NADPH oxidase

NQO1 - NAD (P) H quinona oxidoredutase-1

Nrf-2 - Fator nuclear eritroíde 2 relacionado ao fator 2

$O_2\bullet^-$ - Ânions superóxido

$ONOO^-$ – Peroxinitrito

OXPHOS – Fosforilação oxidativa

PARK2 – "*Parkin RBR E3 Ubiquitin Protein Ligase"*

PDK2 – Quinase piruvato desidrogenase 2

PGC-1α e **PGC-1β** - Co-ativadores α/β ativados pelo proliferador de peroxissomo α/β

PGE2 - Prostaglandinas E2

PI3K - Fosfatidilinositol-4,5 bisfosfato 3-quinase

PKC - Proteína quinase C

POT1 – Proteína de proteção telomérica 1

pRB - Proteína retinoblastoma

Rac1 / 2 – Substrato relacionado com Ras 1/2 de toxina botulínica C3

RAP1 – Proteína ativadora repressora 1

RNA - Ácido ribonucleico

ROS - Espécies reativas de oxigênio

SA-β-gal - β-galactosidase

SASP - Fenótipo secretório associados a senescência

SCO2 – Proteína de montagem citocromo c oxidase

SFK - Src família quinases

Sirtuins – Regulador silencioso de informação

SOD - Superóxido dismutase

TERC – Componente RNA da telomerase

TERT - Telomerase transcriptase reversa

TIN2 – TRF1 e TRF2 proteína de interação nuclear 2

TIFs – Focos induzidos por disfunção telomérica

Tnf-α – Fator de necrose tumoral

TPP1 – Tripeptidil-peptidase 1

TRF1 – Fator de ligação de repetição telomérica 1

TRF2 – Fator de ligação de repetição telomérica 2

ULK1 – "*Uncoordinated-51-like kinase 1*"

VSMCs - Células de músculo liso vascular

VCAM-1 - molécula 1 de adesão de células vasculares

γH2AX – Forma fosforilada da histona H2AX

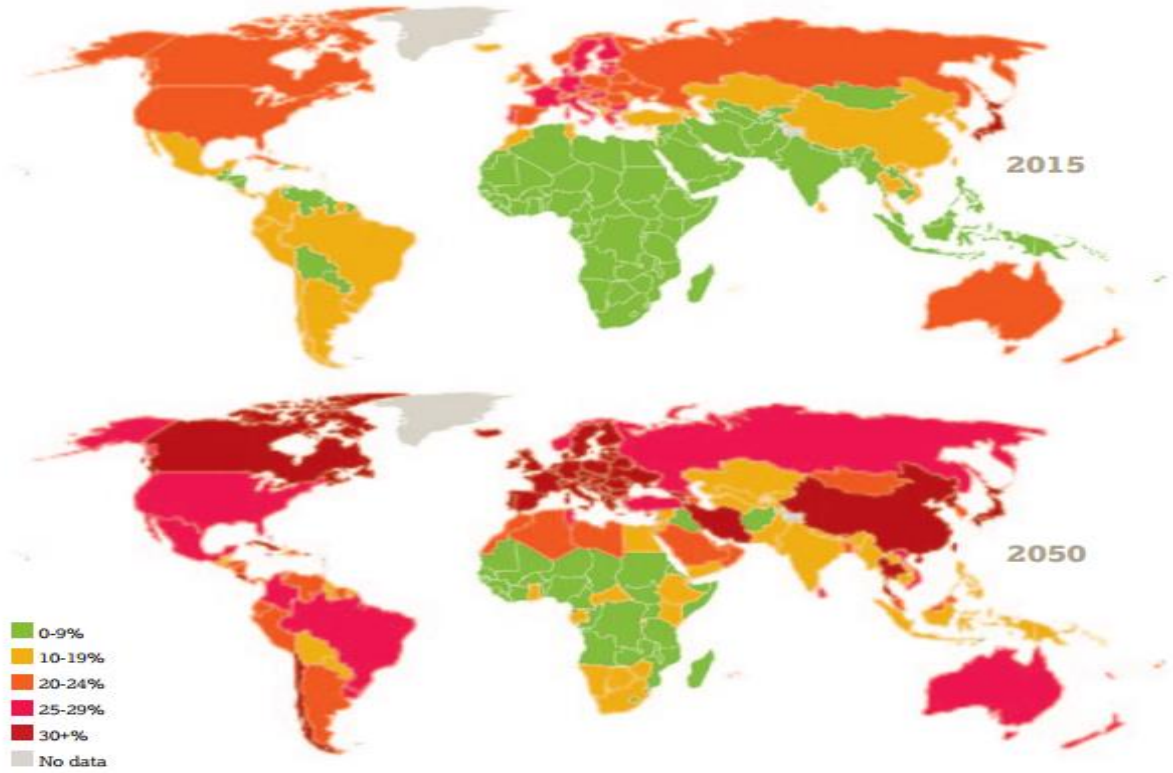
SUMÁRIO

1. INTRODUÇÃO

1.1 ENVELHECIMENTO

A população mundial acima dos 60 anos vai crescer exponencialmente nas próximas décadas, saindo de 12%, dados de 2015, para 22% em 2050 (Figura 1) (UN, 2015). Além de seus efeitos negativos sobre os custos para fundos de aposentadoria, um aumento da idade média vai amplificar o encargo econômico para os custos de saúde. Países em desenvolvimento como Brasil, Índia e China precisarão de 170 anos para se adequar a mudança na pirâmide etária, 20 anos a mais que os países desenvolvidos, como a França e os Estados Unidos (WHO, 2015).

Figura 1. Um mundo envelhecido - População acima dos 60 anos, em 2015 e 2050.



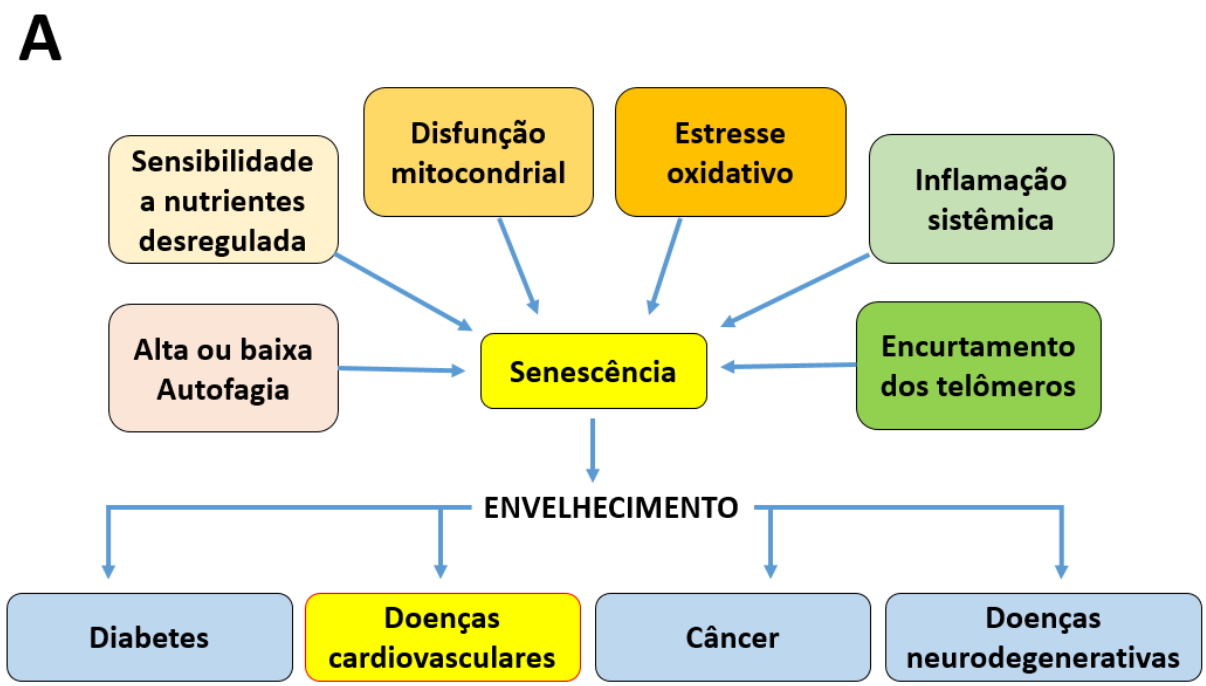
Fonte: Nações Unidas, 2015.

Entretanto, apesar do aumento da expectativa de vida da população, não necessariamente o indivíduo apresenta uma melhora na sua qualidade de vida (Figura x). Doenças como câncer, diabetes, doenças neurodegeneratives e cardiovasculares tem sua prevalência aumentada com a idade. Em 2012, 68% das mortes foram relacionadas com

estas doenças, destacando as doenças cardiovasculares (DCVs), correspondendo a 46% deste total (WHO, 2012).

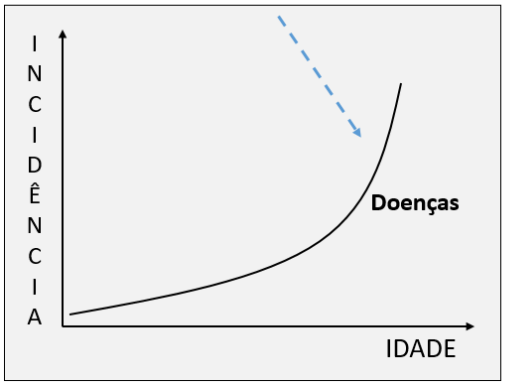
O envelhecimento é um processo universal, multifatorial, caracterizado por um declínio gradual das funções fisiológicas, que ocorre a nível molecular, celular e tecidual (CAMPISI, 2013), englobando uma serie de mecanismos como autofagia, disfunção mitocondrial, encurtamento dos telômeros, estresse oxidativo, processo inflamatório e o metabolismo celular (LÓPEZ-OTÍN *et al.*, 2013). A desregulação nesses mecanismos leva a célula a um estado senescente, no qual contribuem para o envelhecimento, e consequentemente, para as doenças associadas ao envelhecimento (Figura 2A) (RIERA *et al.*, 2016). Embora muitas teorias tenham sido propostas para explicar o processo de envelhecimento, nenhuma delas parecem ser totalmente satisfatória.

Figura 2. Aspectos moleculares do envelhecimento e as doenças relacionadas com a idade.



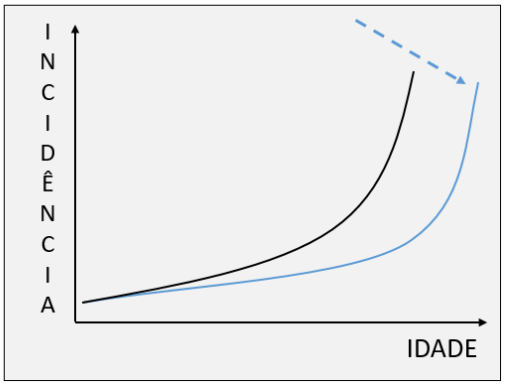
B

Doenças relacionadas com a idade aumenta exponencialmente:
Isso não é coincidência!



Tratando o envelhecimento

Prolongando os anos saudáveis de vida
Melhorando a longevidade e a saúde



Fonte: autor, 2017.

Buscar entender como a idade contribui para o surgimento das doenças relacionadas com o envelhecimento é fundamental para direcionar terapias contra esse processo, a fim de evitar o surgimento dessas doenças, estabelecendo um envelhecimento saudável (Figura 2B).

1.2 SENESCÊNCIA – APOSENTADORIA CELULAR

Senescência é o estado celular no qual ocorre a interrupção do ciclo celular, mas é mantida sua atividade metabólica (CAMPISI e D'ADDA DI FAGAGNA, 2007). Células senescentes secretam uma variedades de agentes pró-inflamatórios como citocinas, interleucinas e fatores de crescimento, no qual são conhecidos como “fenótipo secretório associados a senescência” (SASP) (ZIEGLER *et al.*, 2015).

As células senescentes são geralmente removidas pelo sistema imune, no entanto, em consequência da imunossenescência (senescência da linhagem imune), elas começam a se acumular com a idade (SAGIV e KRIZHANOVSKY, 2013; AUNAN *et al.*, 2016). Acredita-se que os aumentos nos mediadores pró-inflamatórios são inicialmente um mecanismo de "limpeza" das células senescentes, mas com a imunossenescência, o estímulo gerado pelas células senescentes não é capaz de recrutar células funcionais suficientes do sistema imune, processo que tem um efeito negativo sobre o envelhecimento e as doenças relacionadas à idade (GRUVER *et al.*, 2007; VICENTE *et al.*, 2016).

Além disso, há um limite feito pela senescência em linhagens de células-tronco "exaustão de células-tronco", resultando na diminuição de seu potencial regenerativo (COLLADO *et al.*, 2007; OH *et al.*, 2014). Esses dois pontos chave: acumulação de células senescentes e perda da função de linhagens regenerativas, contribuem para o envelhecimento simultaneamente.

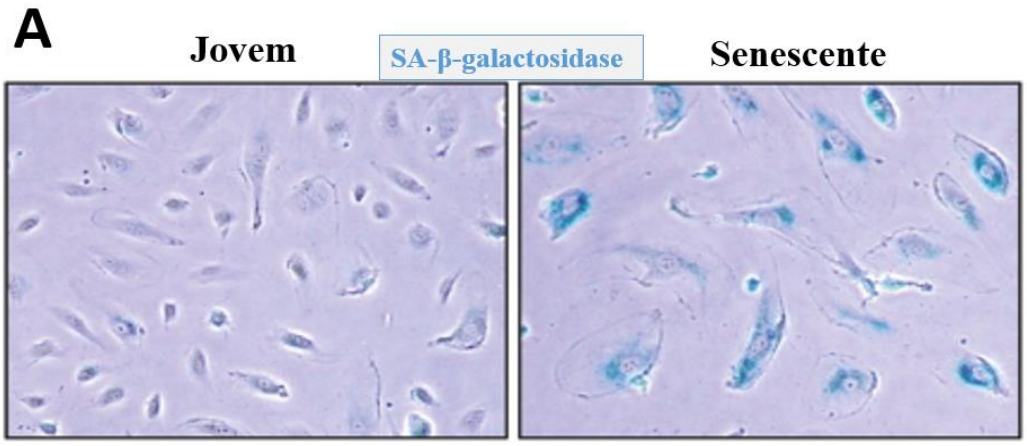
Vários fatores levam à senescência, e uma delas é a divisão celular, com encurtamento de telômeros, chamada senescência replicativa (HARLEY *et al.*, 1990; BERNADOTTE *et al.*, 2016). A senescência também pode ser induzida por estresse, como estresse oxidativo e inflamação, levando a danos no DNA, ativação de oncogenes e mudanças na cromatina (BURTON e KRIZHANOVSKY, 2014). Outra via que leva à senescência, é a disfunção mitocondrial, um processo que diminui o suprimento de energia celular, levando a célula a diminuir a sua atividade metabólica (WILEY *et al.*, 2016; YUE e YAO, 2016). Além disso, a deficiência nas vias da autofagia também leva a célula à senescência através da acumulação de "lixo" celular, que é tóxico para a célula (GEWIRTZ, 2013).

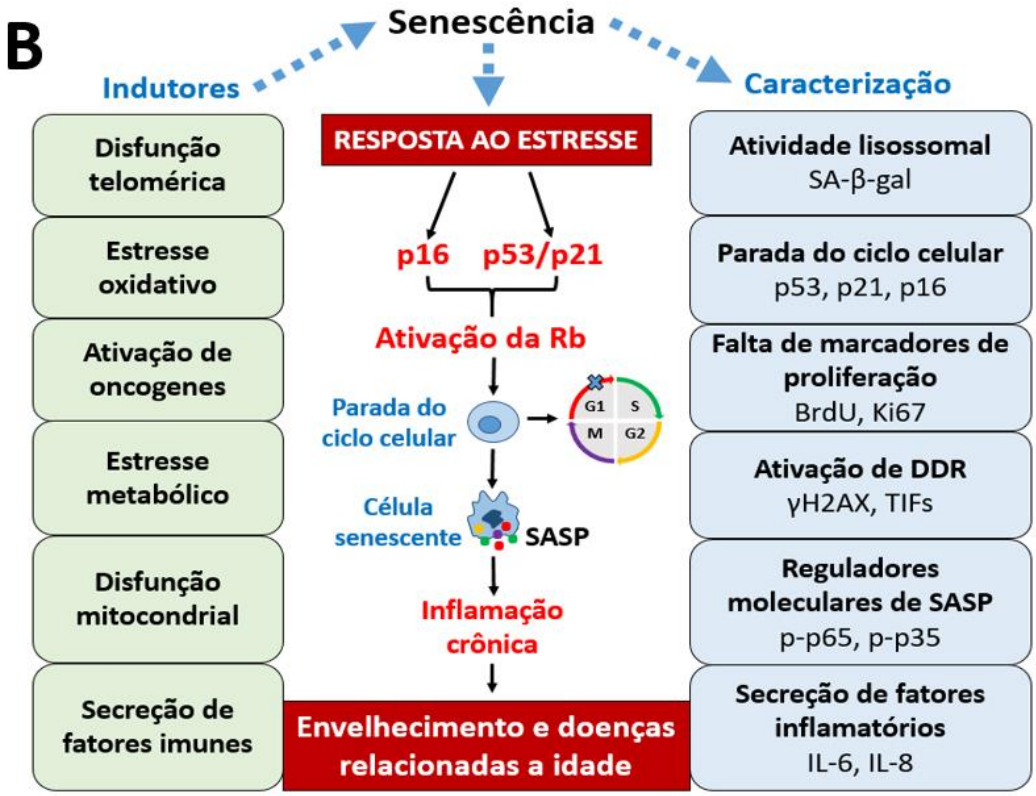
O estágio de senescência é controlado majoritariamente por duas vias principais: p53 / p21 e p16 / pRB. Ambos os caminhos são complexos e possuem vários reguladores, mas ainda não totalmente esclarecidos (LIN *et al.*, 1998; CAMPISI, 2013; MUNOZ-ESPIN e SERRANO, 2014). De modo geral, em resposta ao dano no DNA (DDR), p53 é estimulado e induz a expressão de p21, um inibidor de quinase dependente de ciclina (CDK). Em consequência da supressão da atividade de CDK, a proteína retinoblastoma (pRB) é ativada. A p16, outro inibidor de CDK, também impede a fosforilação de pRB, conduzindo à inativação de pRB (CAMPISI e D'ADDA DI FAGAGNA, 2007; LANIGAN *et al.*, 2011; MUNOZ-ESPIN e SERRANO, 2014). Assim, o pRB desempenha um papel central na senescência e a sua atividade é principalmente atribuída à sua capacidade de ligar e inativar fatores de transcrição da família E2F (fator E2), que induz proteínas do ciclo celular e fatores de replicação do DNA necessários para o crescimento celular (LANIGAN *et al.*, 2011). Desta forma, há uma regulação recíproca entre a sinalização p53 / p21 e p16 / pRB, no entanto, estas vias podem induzir a senescência de forma independente (CAMPISI e D'ADDA DI FAGAGNA, 2007). De fato, células com p16 positivas de ocorrência natural diminuem a duração da vida saudável em ratos (BAKER *et al.*, 2016).

Morfologicamente, as células senescentes são caracterizadas pelo aumento de seu volume e, se aderentes, adotam uma morfologia achatada, porém, não há marcador exclusivamente para o estado senescente (CAMPISI, 2013). O primeiro marcador a ser utilizado foi a detecção de senescência associada à atividade de β-galactosidase (Figura 3A) (SA-β-gal) (Figura 3) (DIMRI *et al.*, 1995), no qual indica um aumento da atividade lisossômica da β-galactosidase (ALTHUBITI *et al.*, 2014).

Recentemente, vários marcadores moleculares foram desenvolvidos e sua associação com SA-β-gal é o padrão-ouro para confirmar a fase de senescência. Estes marcadores representam proteínas de paragem do ciclo celular (p16, p21, p53), falta de marcadores de proliferação (Ki67, BrdU), expressão de fatores de secreção (IL-6, IL-8), ativação de vias de regulação do fenótipo secretório (p-p65 ou P-p38), alterações na cromatina (HP1, Hira) e ativação da DDR (γH2AX, TIFs) (Figura 3B) (ADAMS, 2009; BURTON e KRIZHANOVSKY, 2014).

Figura 3. Senescência e envelhecimento.





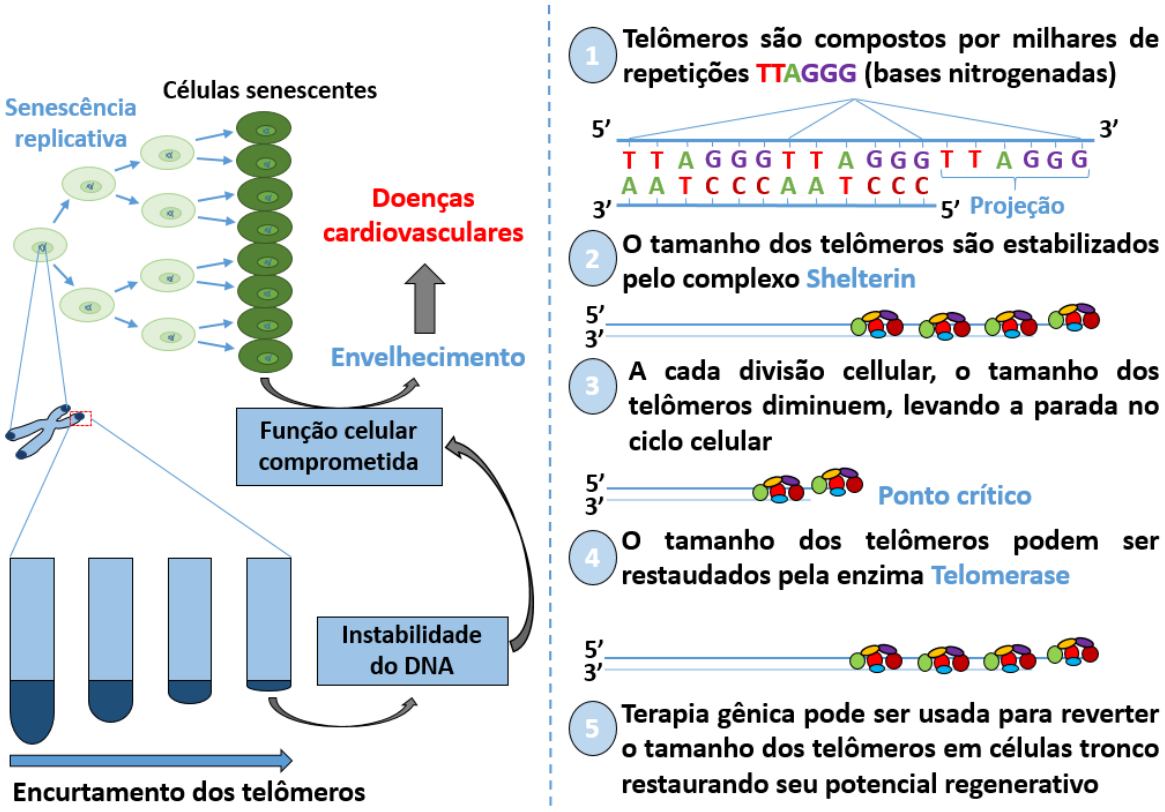
Fonte: A) (MUN GI e BOO YC, 2010). B) autor, 2017.

O aumento no número de células cardíacas, musculares, endoteliais e progenitoras endoteliais estão implicadas na disfunção cardiovascular, levando a progressão de várias doenças, como hipertensão, arteriosclerose, insuficiência cardíaca e derrame. Portanto, terapias que visem retardar ou até mesmo reverter o processo de senescência tem sido propostas para o tratamento dessas doenças (WANG e BENNETT, 2012; TCHKONIA *et al.*, 2013; BAKER *et al.*, 2016; DAVALLI *et al.*, 2016; ROSS *et al.*, 2016).

1.3 TELÔMEROS – O RELÓGIO BIOLÓGICO

Uma das características do envelhecimento molecular é o encurtamento dos telômeros com o advento da idade (LÓPEZ-OTÍN *et al.*, 2013). Os telômeros, conhecidos como relógio biológico, compreendem milhares de sequências de nucleotídeos no final de cada cromossomo. No lado 3 ', a sequência corresponde a TTAGGG (9-15 kb, em seres humanos) (FYHRQUIST *et al.*, 2013). Nas células somáticas, após cada divisão celular, parte dessas bases são perdidas no processo, promovendo o encurtamento dos telômeros (HARLEY *et al.*, 1990). Assim, estima-se um número finito para as divisões celulares, e depois disso, as células tornam-se senescentes (Figura 4) (HAYFLICK e MOORHEAD, 1961).

Figura 4. O papel e função dos telômeros no envelhecimento.



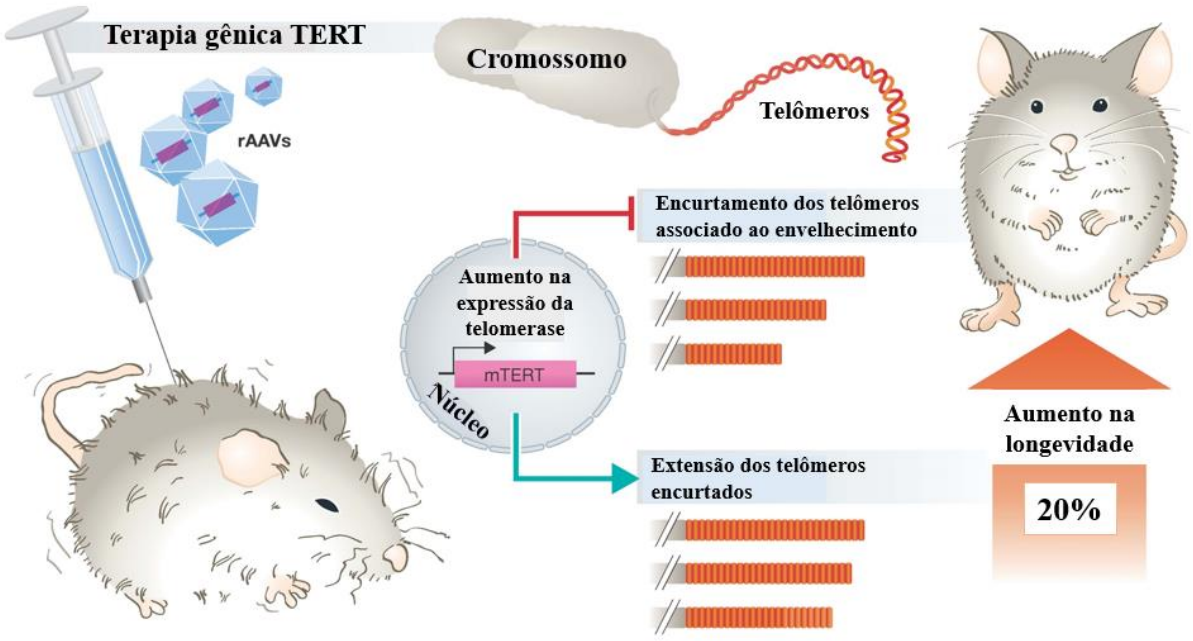
Fonte: autor. 2017.

Associado aos telômeros, há um complexo shelterin formado por proteínas e fatores de transcrição. Este complexo compreende um conjunto de seis subunidades com funções distintas, que tem participação essencial para a proteção cromossômica (MACIEJOWSKI e DE LANGE, 2017). Eles são: fator de ligação de repetição telomérica 1 (TRF1), fator de ligação de repetição telomérica 2 (TRF2), proteína ativadora repressora 1 (RAP1), TRF1 e TRF2 proteína de interação nuclear 2 (TIN2), tripeptidil-peptidase 1 (TPP1) e proteína de proteção telomérica 1 (POT1) (ARMSTRONG e TOMITA, 2017). TRF1 e TRF2 se ligam diretamente às repetições teloméricas de cadeia dupla, enquanto POT1 reconhece a cadeia telomérica no ramo 3 '. TIN2 liga-se a TRF1 e TRF2. TIN2 também recruta o heterodímero TPP1-POT1, reduzindo diferentes proteínas do complexo shelterin para organizar a porção final dos telômeros. O RAP1 é recrutado para os telômeros pela TRF2. Além disso, o RAP1 também pode ligar ao longo de braços cromossómicos que regulam a transcrição de genes (HOHENSINNER *et al.*, 2011).

Os telômeros participam na manutenção do genoma e promovem estabilidade no processo de replicação, evitando recombinação indesejadas e fusão cromossômica (MURAKI *et al.*, 2012; BLACKBURN *et al.*, 2015). Quando o tamanho crítico do telômeros é atingido, o sistema de reparo do DNA ativa vários pontos de checagens celular (WRIGHT e SHAY, 1992; HAYASHI *et al.*, 2012). Já foram identificados dois pontos de controle que limitam a vida celular em resposta à disfunção dos telômeros: O primeiro posto de controle (M1, primeira fase da mortalidade) é caracterizado por uma parada completa do ciclo celular, conhecida como senescência, sendo dependente da ativação de p53 (FYHRQUIST *et al.*, 2013). Células com mutação no gene p53 podem continuar a se dividir mesmo com o tamanho crítico dos telômeros atingidos (SHAY e WRIGHT, 2005; MACIEJOWSKI e DE LANGE, 2017). Se a célula continua a se dividir e, consequentemente, os telômeros continuam a diminuir de tamanho, um novo posto de controle é ativado (M2, segundo estágio da mortalidade), chamado de crise. Este ponto é independente de p53 e é caracterizado por alta instabilidade cromossómica e morte celular (HAYASHI *et al.*, 2015).

Em algumas linhagens celulares, como as células-tronco, o encurtamento dos telômeros pode ser restaurado pela enzima telomerase transcriptase reversa (TERT), juntamente com seu RNA componente (TERC) (NANDAKUMAR e CECH, 2013). Ambos são regulados pelo complexo shelterin (HOCKEMEYER e COLLINS, 2015). Vários estudos relataram que a indução da atividade de TERT em células somáticas reverte várias características do envelhecimento, como a senescência (Figura x) (BÄR e BLASCO, 2016; YEH e WANG, 2016). A capacidade das células tronco pluripotentes embriogênicas ou induzidas (iPSC) de se replicar indefinidamente deve-se a uma alta expressão de TERT funcional e TERC nessas populações celulares (YANG *et al.*, 2008; MARIÓN E BLASCO, 2010).

Figura 5. Terapia gênica – Telomerase.



Fonte: (BOCCARDI e HERBIG, 2012).

Além disso, o encurtamento de telômeros em linfócitos circulantes, usado como marcador indireto de células progenitoras circulantes, foi identificado como um alarme de início para doenças cardiovasculares (PANAYIOTOU *et al.*, 2010). Portanto, há uma grande evidência de que o combate ao encurtamento dos telômeros tem efeitos benéficos sobre o sistema cardiovascular, através da desaceleração ou mesmo da reversão da senescência celular (BÄR *et al.*, 2014; NAZARI-SHAFTI e COOKE, 2015; ZUREK *et al.*, 2016).

1.4 ENVELHECIMENTO – IMPLICAÇÕES SOBRE O SISTEMA CARDIOVASCULAR

O envelhecimento cardiovascular é definido como uma degeneração progressiva dependente da idade, o que torna o coração e os vasos mais vulneráveis ao estresse, contribuindo para o aumento da mortalidade e morbidade (CHIAO e RABINOVITCH, 2015). Em particular, o envelhecimento vascular é caracterizado por alterações moleculares, estruturais, celulares e fisiológicas, sendo o envelhecimento o principal fator de risco independente na patogênese das doenças cardiovasculares (DAI *et al.*, 2012; PANENI *et al.*, 2017).

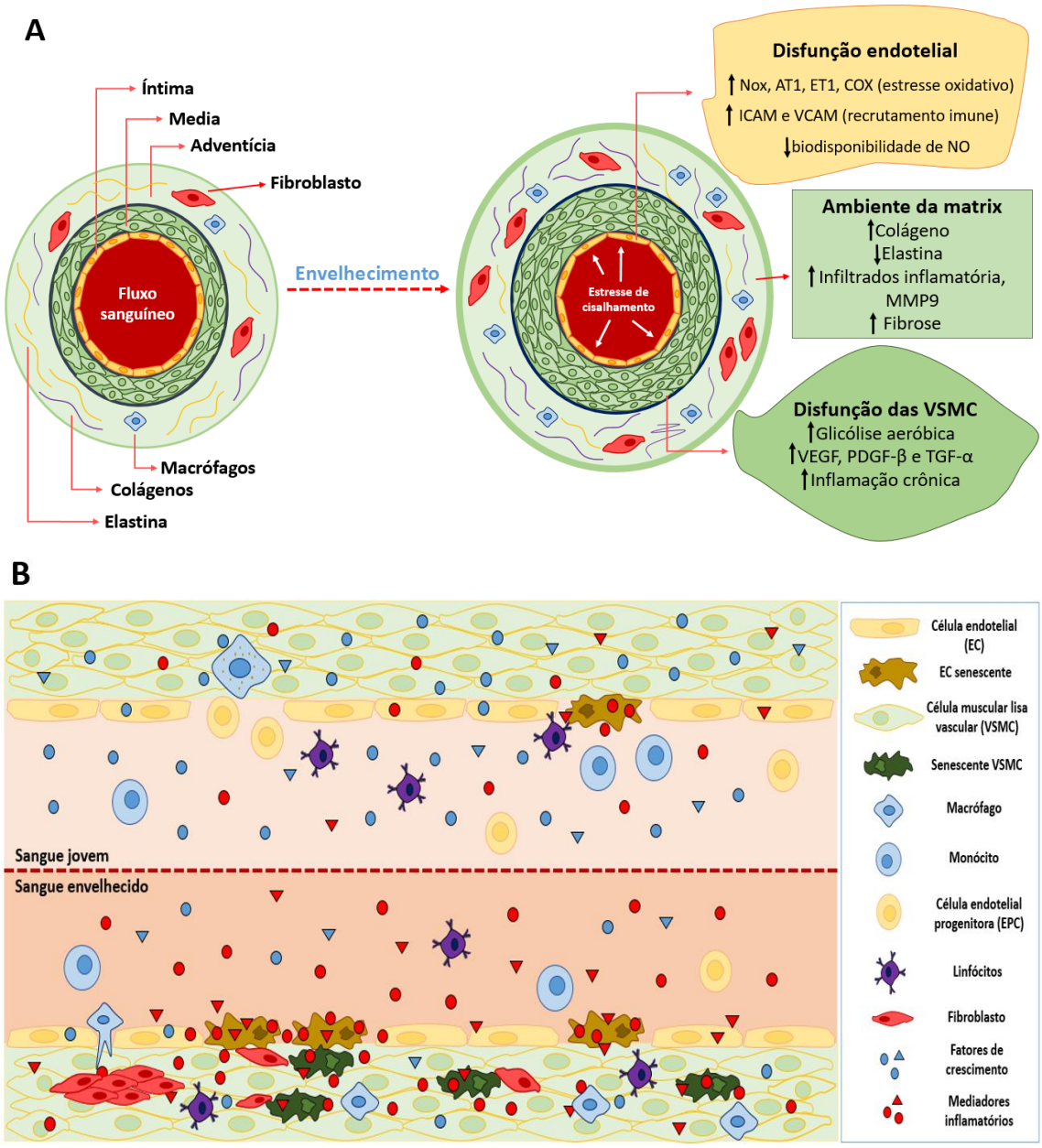
O envelhecimento vascular leva ao espessamento da camada íntima e da camada média (remodelamento vascular), bem como à perda gradual da elasticidade arterial,

resultando em rigidez vascular (LEE e OH, 2010; VAN VARIK *et al.*, 2012). Aumento de colágeno e diminuição do conteúdo de elastina, promovido pelo menos em parte pela idade, além de proteínas glicosiladas aumentadas, atividade da metaloproteinases (MMPs) da matriz e estímulos sistêmicos como a sinalização da angiotensina II, estão relacionados à rigidez vascular (Figura 5.A) (WANG e SHAH, 2015). As células endoteliais (ECs) envelhecidas e as células de músculo liso vascular (VSMCs) também mostram uma maior secreção de citocinas pró-inflamatórias, resultando em inflamação vascular persistente (WANG e BENNETT, 2012).

Um dos principais marcadores da função vascular é a produção de óxido nítrico (NO) (RIBEIRO *et al.*, 2016). No vaso, a sua síntese é feita principalmente pela sintase de óxido nítrico endotelial (eNOS), sendo o envelhecimento associado a uma diminuição da produção de NO (UNGVARI *et al.*, 2010; VALERIO e NISOLI, 2015). Em camundongos com envelhecimento precoce, a disfunção endotelial associada à idade aórtica está ligada à disfunção eNOS (NOVELLA *et al.*, 2013). O aumento na produção de espécies reativas de oxigênio (ROS) e subsequente inativação do NO é um mecanismo importante envolvido na diminuição do relaxamento do vaso dependente do endotélio, levando à rigidez e inflamação vascular (SEALS *et al.*, 2014; VENDROV *et al.*, 2015).

Assim, o remodelamento vascular, por envelhecimento ou condições patológicas, é acompanhado por estresse oxidativo e inflamação, levando a um aumento das células senescentes nesses tecidos (Figura 5.B) (WU *et al.*, 2014). As células endoteliais têm importância fundamental no desenvolvimento do remodelamento vascular, sendo a disfunção endotelial alvo de terapias contra DCVs, tais como hipertensão, aterosclerose e insuficiência cardíaca (STEYERS e MILLER, 2014; DONATO *et al.*, 2015; PANTH *et al.*, 2016).

Figura 6. Envelhecimento cardiovascular.



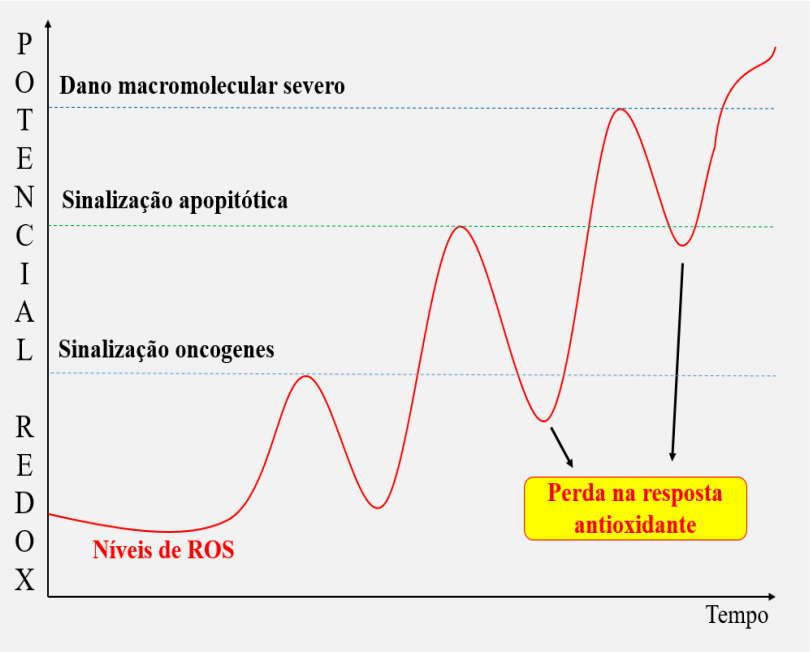
Fonte: autor, 2017.

1.5 O PAPEL DAS ROS E DO ESTRESSE OXIDATIVO – UM MEDIADOR NECESSÁRIO

De acordo com a teoria dos radicais livres do envelhecimento proposta por Harman em 1956, as ROS leva a dano oxidativo em biomoléculas celulares, contribuindo para o declínio da função fisiológica com o envelhecimento (HARMAN, 1956). Embora uma série de revisões e evidências relatar os efeitos deletérios dos ROS no envelhecimento (WU *et al.*, 2014; SKIBSKA e GORACA, 2015), estudos recentes em animais geneticamente modificados desafiam o papel dos ROS no envelhecimento (LAPOINTE e HEKIMI, 2010). Desse modo, as ROS parece ter um duplo efeito.

Inicialmente, como ativador de uma resposta compensatória homeostática que aumenta com a idade para manter a sobrevivência através da ativação de vários mecanismos de defesa, além de estimular a proliferação celular e, a partir de certo limite, como fator que ao invés de aliviar, agrava os danos associados ao envelhecimento (Figura 6) (HEKIMI *et al.*, 2011; HEKIMI *et al.*, 2016).

Figura 7. Efeito dual das ROS.



Fonte: autor, 2017.

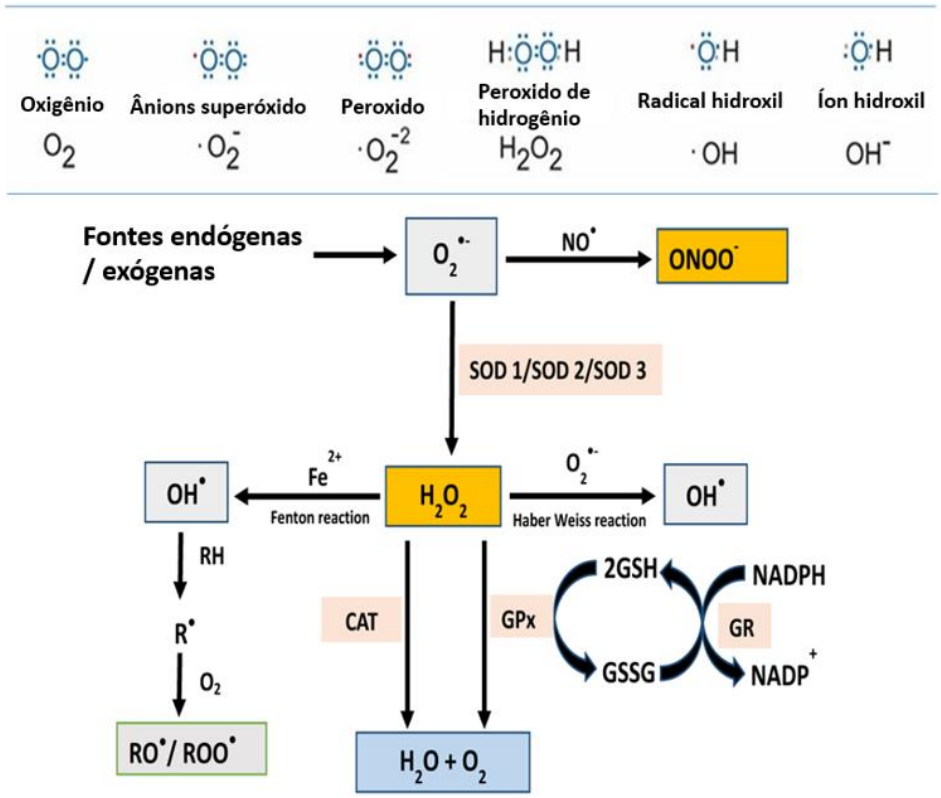
Existem várias fontes de ROS em mamíferos, incluindo a respiração mitocondrial, ciclooxigenase e lipoxigenase, citocromo p450s, xantina oxidase, NADPH oxidase, NO sintase, peroxidase, retículo endoplasmático e outras hemoproteínas (HOLMSTROM e FINKEL, 2014; CERVANTES GRACIA *et al.*, 2017).

Muitas espécies de ROS têm elétrons desemparelhados, chamados de radicais livres. Neste grupo incluem-se os ânions superóxido ($O_2^{\bullet -}$), o radical hidroxil ($HO^{\bullet}$), o óxido nítrico (NO) e os radicais lipídicos. Outras espécies de oxigénio reativo tais como peróxido de hidrogénio (H_2O_2), peroxinitrito ($ONOO^-$) e ácido hipocloroso (HOCl) não são radicais livres mas têm efeitos oxidantes que contribuem para o stress oxidativo (CAI e HARRISON, 2000; BROWN e GRIENDLING, 2015).

O equilíbrio basal nos níveis de ROS é mediado pela atividade de um conjunto de complexos enzimáticos e não-enzimáticos com a função de desintoxicação celular,

chamados coletivamente de antioxidantes (PENG *et al.*, 2014). O fator nuclear eritroíde 2 relacionado ao fator 2 (Nrf-2), um fator de transcrição, é o principal regulador do sistema enzimático antioxidante, incluindo transcrição de enzimas antioxidantes e desintoxicadoras de fase II tais como superóxido dismutase (SOD), catalase (CAT) glutationa peroxidase (GPx), glutationa redutase (GR), hemeoxigenase-1 (HO-1) e NAD (P) H quinona oxidoredutase-1 (NQO1). De modo geral, este sistema é o principal sistema de defesa que neutraliza a produção de ROS *in vivo* (Figura 7) (KENSLER *et al.*, 2007; GORRINI *et al.*, 2013).

Figura 8. As ROS e o sistema antioxidante.



Fonte: adaptado de (AJUWON *et al.*, 2015; BIOTEK, 2014), disponível em < https://www.biotek.com/resources/single.html?newsid=10592 >.

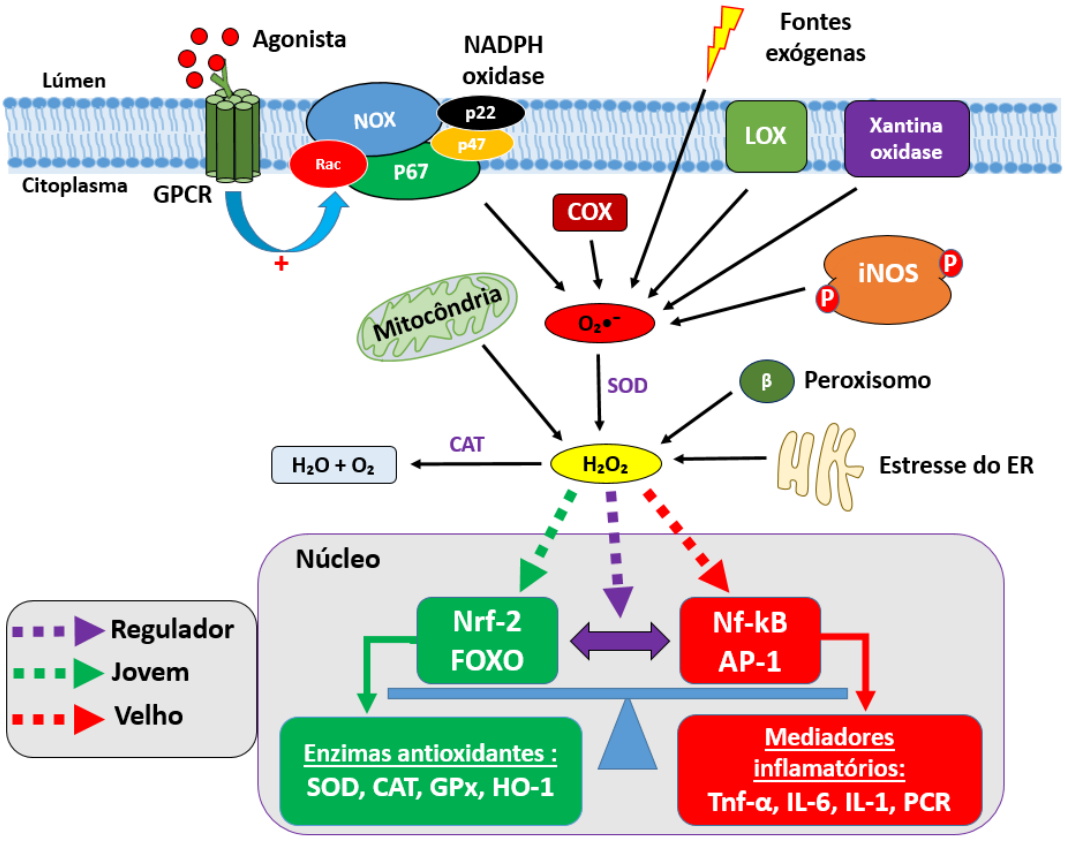
Um desequilíbrio para o lado pró-oxidante leva ao estado fisiológico conhecido como estresse oxidativo (CONTI *et al.*, 2015).

A NADPH oxidase (Nox) é uma importante fonte de ROS no sistema cardiovascular (SANTILLO *et al.*, 2015). Existem sete isoformas Nox: Nox1, Nox2, Nox3, Nox4, Nox5, Duox1 e Duox2. Todos os Nox são proteínas transmembranares que possuem um sítio catalítico (Nox) e um complexo proteico regulatório (MONTEZANO

E TOUYZ, 2013). As Isoformas 1, 2, 4 e 5 são expressas em vários tecidos, incluindo o coração e os vasos. Nox2 e Nox4 são super-expressos no tecido vascular de ratos velhos (SCHRAMM *et al.*, 2012). O protótipo do grupo é o Nox2 que é composto por 6 subunidades: p47phox, p67phox, p40phox e Rac1 / 2 que são proteínas reguladoras citosólicas, p22phox que é uma proteína reguladora de membrana e gp91phox, que é uma subunidade catalítica presente na membrana (LASSÈGUE *et al.*, 2012).

O complexo de Nox é regulado positivamente pelo fator de necrose tumoral (Tnf-α) (SRIRAMULA e FRANCIS, 2015) como pela ativação do receptor AT1 pela angiotensina II (MONTEZANO *et al.*, 2014). Assim, com o aumento na produção de ROS, o Nrf-2 começa a ter sua atividade inibida pelo *crosstalk* com fator nuclear kappa B (Nf-kB), responsável pelo aumento dos níveis de Tnf-α, gerando um ciclo vicioso (LIU *et al.*, 2008). À medida que as ROS são produzidos, a liberação de Tnf-α aumenta, agravando o estresse oxidativo. Esta mudança na expressão de Nrf-2 para Nf-kB parece ser gradual, acompanhando o envelhecimento (Figura 8).

Figura 9. Potencial redox controla o destino celular.



Fonte: autor, 2017.

A principal fonte de ROS durante o envelhecimento é a mitocôndria (DAI, CHIAO, *et al.*, 2014). Harman em 1972, revisou sua teoria sobre os radicais livres após a descoberta de que as mitocôndrias transformaram o oxigênio em água, processo que, quando deficiente, resulta em uma alta produção de ânions superóxido, aumentando os níveis mitocondriais de ROS (mtROS), que levam à acumulação de dano ao DNA mitocondrial (mtDNA), conduzindo à disfunção dessa organela, resultando no envelhecimento (HARMAN, 1972). No entanto, há evidências recentes envolvendo mtROS usando modelos de animais de longevidade que rejeitam, pelo menos em parte, a ideia original da teoria mitocondrial do envelhecimento (HEKIMI *et al.*, 2016).

Estudos com *C. elegans* relatam que, por supressão do gene SOD2, o aumento de mtROS parece prolongar sua expectativa de vida (VAN RAAMSDONK e HEKIMI, 2009). Em *Drosophila*, mtROS da cadeia de transporte de elétrons também parece ter um efeito positivo na sua longevidade (SCIALÒ *et al.*, 2016). Além disso, a super-expressão da catalase aumenta a resistência ao estresse oxidativo, mas não melhora sua longevidade (MOCKETT *et al.*, 2003). Nos vermes, as dietas antioxidantes reduzem seu tempo de vida (PÉREZ *et al.*, 2009). Em ratos, as alterações genéticas que aumentam o mtROS e os danos oxidativo não aceleram o envelhecimento, embora induzam o aparecimento de várias doenças relacionadas com a idade (ZHANG *et al.*, 2009). Há evidências de que mtROS e ROS citosólicas têm efeitos opostos, sendo o citosólico mais tóxico para a célula (SCHAAR *et al.*, 2015).

Portanto, o H_2O_2 produzido com proposta benéfica nas mitocôndrias, eventualmente difunde através da membrana mitocondrial (VEAL *et al.*, 2007), atingindo o citoplasma contribuindo para o estresse oxidativo envolvido no envelhecimento, sugerindo que os efeitos das ROS dependem de onde elas estão presentes e de sua concentração (SCHAAR *et al.*, 2015).

Os idosos são mais suscetíveis ao estresse oxidativo devido a uma redução na eficiência de seus sistemas antioxidantes endógenos. Órgãos como o coração, que tem uma taxa de replicação limitada e altos níveis de consumo de oxigênio, são particularmente mais sensíveis a esse fenômeno, o que explica, em parte, uma alta prevalência de doenças cardiovasculares no envelhecimento (CONTI *et al.*, 2016). Por outro lado, nas células endoteliais, as ROS derivado do complexo NADPH oxidase induz *in vivo* quinases pró-sobrevivência via proteína quinase ativada por 5’ monofosfato de

adenosina (AMPK), além do efeito adicional de indução de autofagia, melhorando a função vascular em coronárias de ratos idosos (SHAFIQUE *et al.*, 2013). Assim, esta abordagem integra conceitos paradoxais sobre o papel benéfico, deletério ou neutro dos ROS no envelhecimento.

1.6 INFLAMAÇÃO – UMA GUERRA SEM EXÉRCITO

O envelhecimento é acompanhado por um aumento sistêmico de agentes pró-inflamatórios (FRANCESCHI e CAMPISI, 2014). As células senescentes têm a capacidade de libertar agentes pró-inflamatórios (SASP) capazes de atrair células de defesa, que fagocitose as células senescentes (COPPÉ *et al.*, 2010; HAE-OK *et al.*, 2015). No entanto, no envelhecimento, ocorre o esgotamento das células-tronco, reduzindo a capacidade regenerativa do organismo, bem como a produção de células imunológicas, termo conhecido como imunossenescência, permitindo a acumulação de células senescentes no corpo, gerando a predisposição para o início de doenças cardiovasculares (Figura 5.B) (SAGIV e KRIZHANOVSKY, 2013).

Os componentes do SASP incluem agentes tais como Fator Tnf-a, IL-6 e IL-1β (FOUGÈRE *et al.*, 2016). Esses agentes pró-inflamatórios são principalmente regulados por fatores de transcrição sensíveis ao potencial redox, como ativador da proteína-1 (AP-1) e do Nf-kB_(MANEA *et al.*, 2015). A superprodução de ROS é essencial para a ativação de AP-1 e Nf-kB através do estresse de quinases tais como quinases reguladoras de sinal extracelular (ERKs), quinases N-terminais c-jun (JNKs), mitogenos (p38 MAPK), proteína quinase C (PKC), fosfatidilinositol-4,5 bisfosfato 3-quinase (PI3K), Akt e Src família quinases (SFK) (SALLAM e LAHER, 2016).

Isto conduz a uma maior expressão de proteínas alvo inflamatórias tais como a MMP9, a molécula de adesão intercelular-1 (ICAM-1), a molécula 1 de adesão de células vasculares (VCAM-1), a sintase óxido nítrico indutível (iNOS), a ciclooxigenase -2 (COX-2), fosfolipase A2 citosólica (cPLA2) e mediadores pró-inflamatórios tais como TNF-α, IL-1 e IL-6. Muitas destas proteínas inflamatórias ou seus produtos, tais como iNOS, COX e prostaglandinas E2 (PGE2) são fontes proeminentes de ROS (LIN, LIN, *et al.*, 2016; LIN, YANG, *et al.*, 2016; MATZKIN *et al.*, 2016; SALLAM e LAHER, 2016).

Portanto, uma das características fundamentais associadas ao envelhecimento é o *crosstalk* entre estresse oxidativo e inflamação (Figura 8). É necessário ressaltar que

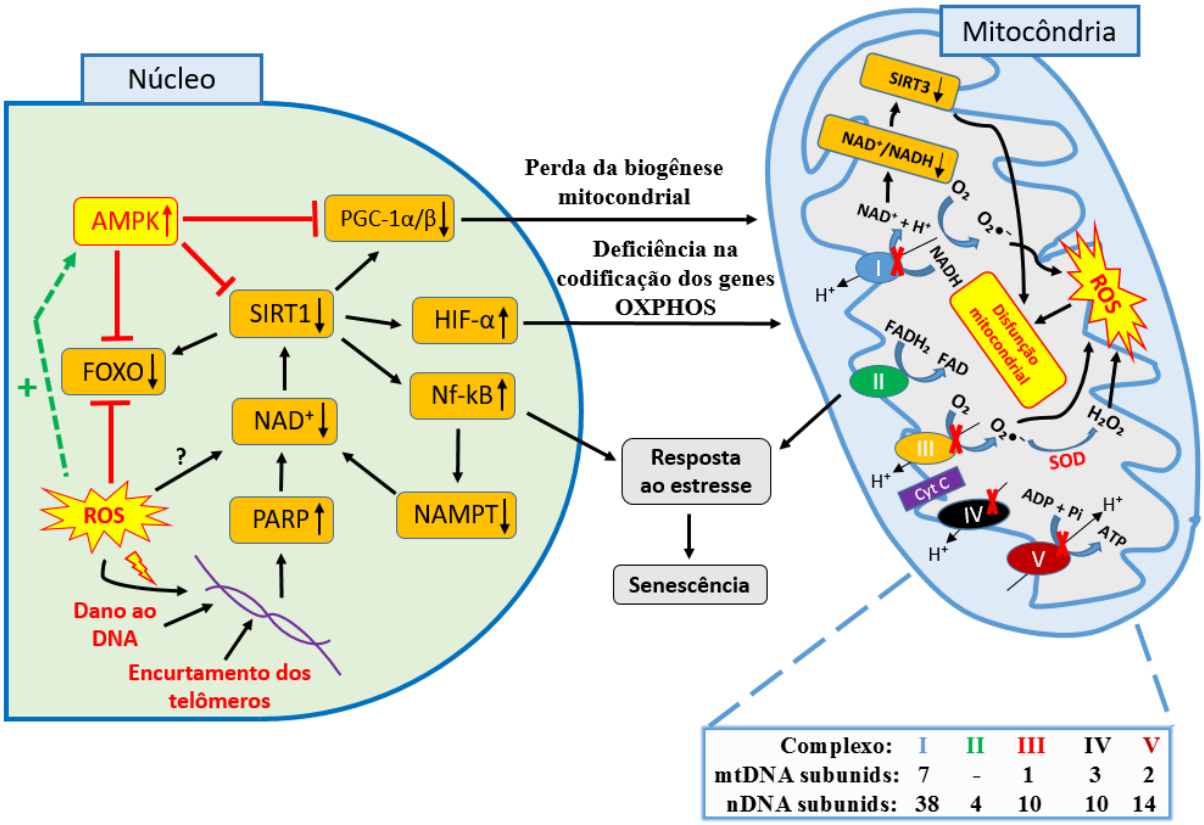
ambos os processos contribuem para a defesa do organismo fisiológico, e no indivíduo jovem, esses processos estão com sua atividade funcional basal.

1.7 DISFUNÇÃO MITOCONDRIAL – UMA FALHA DE COMUNICAÇÃO

As mitocôndrias são consideradas a "central de energia" celular, uma vez que têm a capacidade de gerar trifosfato de adenosina (ATP) através da fosforilação oxidativa (OXPHOS), fornecendo energia química para a sobrevivência e função celular (YUE e YAO, 2016). Além disso, há evidências de que as mitocôndrias desempenham um papel não-energético na regulação do metabolismo, apoptose, imunidade inata e envelhecimento (NUNNARI e SUOMALAINEN, 2012; GONZALEZ-FREIRE *et al.*, 2015; HELD e HOUTKOOPER, 2015).

Apesar da maioria dos genes mitocondriais terem sido transferidos para o genoma nuclear, 13 subunidades essenciais para a atividade OXPHOS permanecem codificadas pelo mtDNA. As outras 76 subunidades são codificadas pelo genoma nuclear, sintetizadas no citoplasma e importadas para as mitocôndrias, necessitando de comunicação funcional entre os dois genomas (RYAN e HOOGENRAAD, 2007; MISHRA e CHAN, 2014). Essa interação funcional é essencial para a saúde mitocondrial e a falha desta comunicação leva à disfunção mitocondrial, diminuindo a síntese de ATP (Figura 9) (GONZALEZ-FREIRE *et al.*, 2015).

Figura 10. Interação núcleo – mitocôndria.



Fonte: autor, 2017.

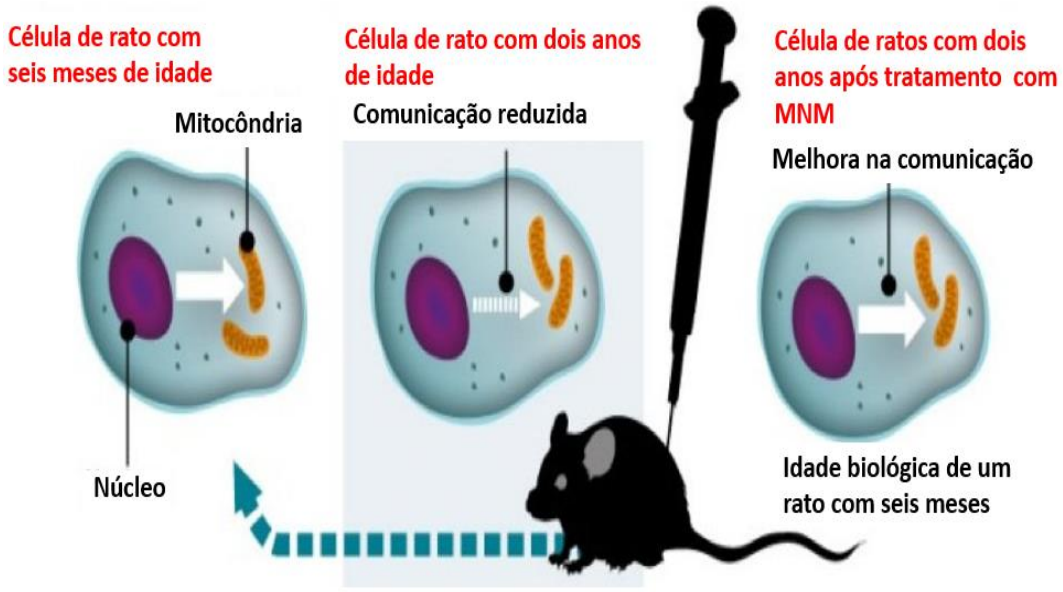
A regulação das mitocôndrias ocorre principalmente por co-ativadores α e β ativados pelo proliferador de peroxissomo α e β (PGC-1α e PGC-1β), que respondem às mudanças no estado nutricional, como a relação NAD^+ / NADH e AMP / ATP (regulados por SIRT1 e AMPK, respectivamente) (FRIEDMAN e NUNNARI, 2014; FANG *et al.*, 2016).

Recentemente, Gomes e colaboradores descreveram um processo de regulação das mitocôndrias via HIF-1α, independente de PGC-1α / β, em resposta à atividade de SIRT1, a qual é controlada por níveis NAD^+ nucleares. Seis horas após a indução da deleção do gene SIRT1 em mioblastos, os níveis de HIF-1α começam a aumentar e após 12 horas ocorre perda de homeostase mitocondrial, embora os níveis de ROS só aumentem após 24 horas do procedimento (GOMES *et al.*, 2013).

No envelhecimento, não há perda nos níveis de SIRT1 no organismo, mas os níveis de NAD^+ diminuem com a idade, levando a uma diminuição na atividade de SIRT1 (IMAI e GUARENTE, 2014). Como resultado, ocorre um estado pseudo-hipóxico, diminuindo a atividade dos complexos I, III, IV e V (codificados pelo genoma nuclear e mitocondrial), mas não do complexo II (codificado pelo genoma nuclear) (RYAN e

HOOGENRAAD, 2007). Para recuperar a atividade dos complexos I, III, IV e V, é necessário restaurar a comunicação do genoma mitocondrial e nuclear, o que é conseguido através da suplementação com NMN (precursor NAD^+). Os tratamentos que restauram os níveis de NAD^+ demonstraram ser benéficos para restaurar a função mitocondrial e vários aspectos relacionados ao envelhecimento em camundongos (Figura 10) (GOMES *et al.*, 2013; IMAI e GUARENTE, 2014; ZHANG *et al.*, 2016), indicando que o envelhecimento é pelo menos em parte, causada por uma falha na comunicação nuclear-mitocondrial. Processo que é dependente do equilíbrio celular energético (GOMES *et al.*, 2013; FANG *et al.*, 2016).

Figura 11. Reparo na comunicação núcleo – mitocôndria.



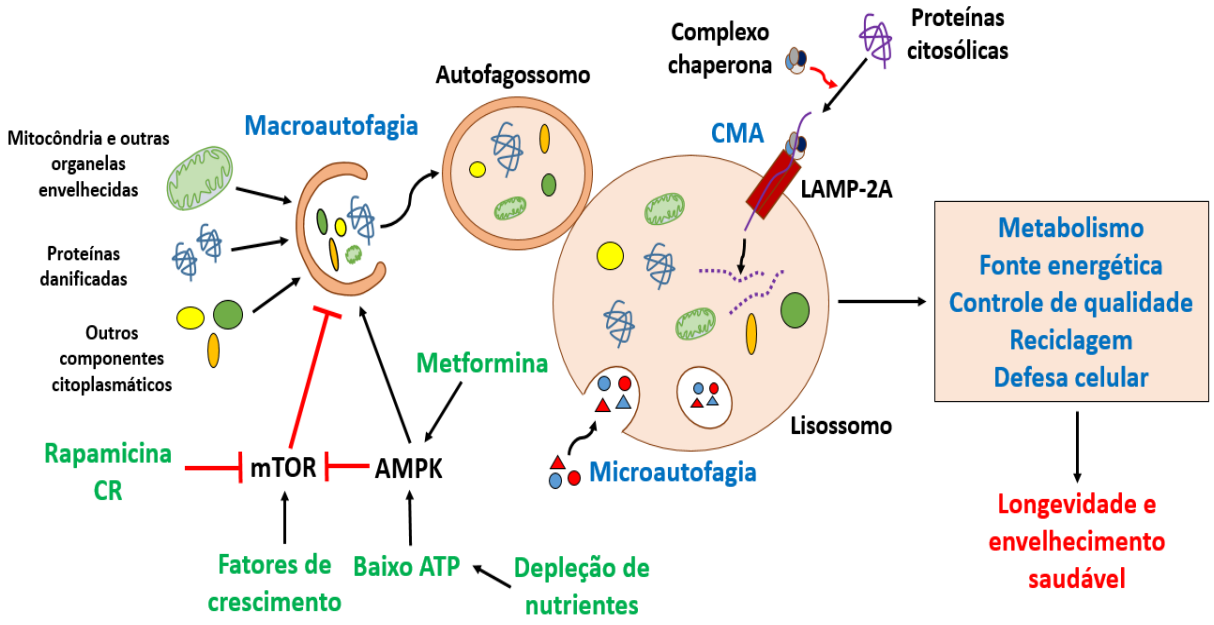
Fonte: (GOMES *et al.*, 2013). Imagem disponível em < http://www.smh.com.au/technology/sci-tech/scientists-find-way-to-make-ageing-clock-stop-ticking-20131219-2zohf >.

1.8 AUTOFAGIA – SECRESTADORES CELULARES

Autofagia ou "auto-alimentação" refere-se ao processo de degradação lisossomal que remove agregados de proteínas, organelas danificadas, substâncias tóxicas e até patógenos (DE DUVE e WATTIAUX, 1966). Este processo é essencial para manter a integridade celular e sua homeostase, fornecendo metabólitos para a sobrevivência celular sob condições de stress (RABINOWITZ e WHITE, 2010). Além disso, ajuda a manter os níveis de energia celular durante limitações de nutrientes através de processos de reciclagem (KIM e LEE, 2014).

Existem três tipos de autofagia atualmente descritos, conhecidos como: macroautofagia, microautofagia e autofagia mediada por chaperonas (CMA). Todas diferem em seus mecanismos e funções (LENOIR *et al.*, 2016). A microautofagia produz invaginações aleatórias na membrana lisossomal, envolvendo componentes citoplasmáticos próximos ao lúmen lisossômico (SANTAMBROGIO e CUERVO, 2011). A CMA atua diretamente através da membrana lisossomal através do receptor específico LAMP-2A (proteína tipo 2 associada a membrana do lisossomo) (LI *et al.*, 2011). A macroautofagia, muitas vezes referida como autofagia, requer a formação de uma membrana dupla (autofagosomo) envolvendo o material a ser degradado, sendo posteriormente fundido ao lisossomo (Figura 11) (RUSSELL *et al.*, 2014).

Figura 12. O papel da autofagia no envelhecimento saudável.



Fonte: autor, 2017.

A autofagia é principalmente regulada negativamente pelo complexo mTOR. Este complexo é ativado em condições ricas em nutrientes, desempenhando um papel fundamental na sensibilidade aos nutrientes (LEE *et al.*, 2012). Em uma condição pobre em nutrientes, outro sensor energético é ativado, o AMPK, que inibe diretamente o mTOR pela sua fosforilação direta, bem como ativa diretamente a ULK1 (família de proteínas ATG) (genes ATG relacionados à autofagia), estimulando a autofagia (RUSSELL *et al.*, 2014). A escassez de nutrientes também regula a autofagia em nível de transcrição

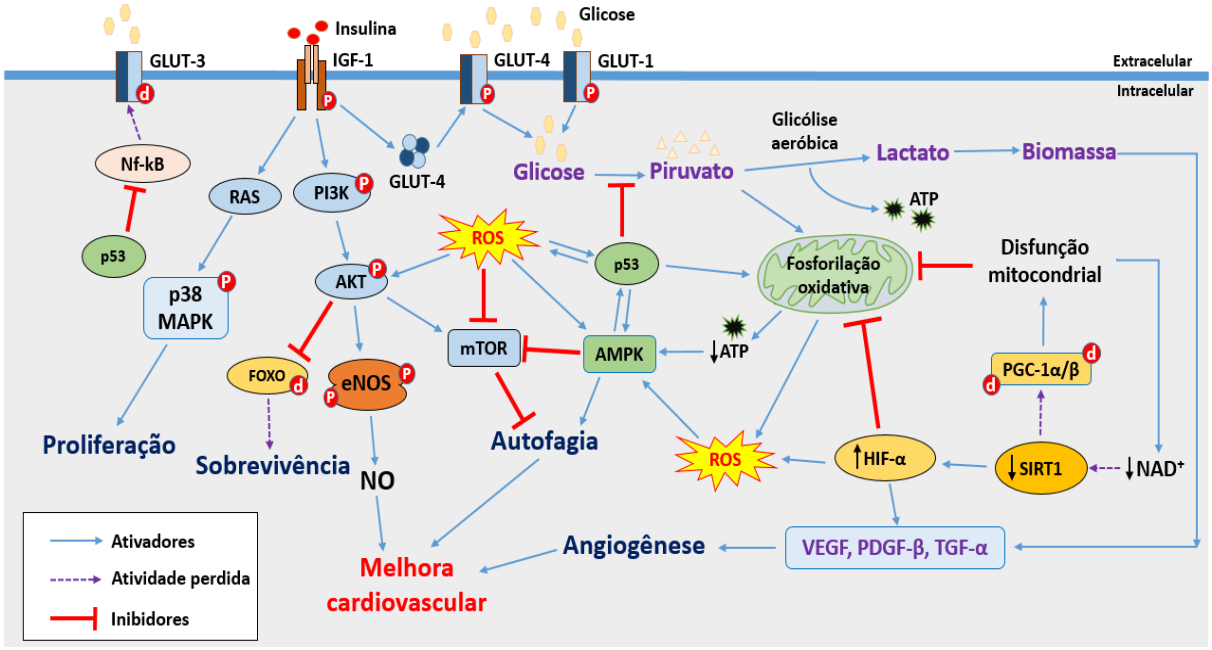
modulando a expressão de genes codificados por ATG, e este mecanismo é mediado pelo menos em parte pelo fator de transcrição FOXO1 (RIEHLE e ABEL, 2014).

No envelhecimento, a autofagia é desregulada ou inoperante, favorecendo a acumulação de "lixo" na célula (SHIRAKABE *et al.*, 2016). A atividade excessiva do complexo mTOR durante o envelhecimento aumenta os agregados de proteínas anormais, estando relacionadas com a gênese das doenças cardiovasculares (XU *et al.*, 2014). Por outro lado, o aumento da autofagia pela inibição de mTOR, leva a um aumento no envelhecimento saudável, melhorando a função cardiovascular e prevenindo as doenças cardiovasculares (MARKAKI e TAVERNARAKIS, 2013; JIA *et al.*, 2014).

1.9 CONTROLE METABÓLICO DO ENVELHECIMENTO – CONECTANDO OS PONTOS

O envelhecimento é caracterizado por uma diminuição no suprimento energético celular (ZIEGLER *et al.*, 2015). Os principais reguladores desse processo são as mitocôndrias como fonte de ATP e os lisossomos, uma organela essencial para a autofagia, um dos mecanismos responsáveis pela geração de energia em tempos de escassez de nutrientes (IKEDA *et al.*, 2014; PHILLIP *et al.*, 2015). Vários mecanismos que melhoram a função desses processos desempenham um papel benéfico no envelhecimento (SEAH *et al.*, 2016; SHIRAKABE *et al.*, 2016). Os mecanismos envolvidos neste processo tem vários reguladores tais como Insulina / IGF-1, mTOR, AMPK e Sirtuins (SOLON-BIET *et al.*, 2015). Outros fatores como ROS e via p53 também parecem ser fundamentais no controle de bioenergético (Figura 12) (SUN *et al.*, 2012; WILEY e CAMPISI, 2016).

Figura 13. Controle metabólico envolvido no sistema cardiovascular.



Fonte: autor, 2017.

A via da insulina / fator de crescimento-1 (IGF-1) controla a sobrevivência, proliferação e processos metabólicos. Este mecanismo é um dos caminhos bem caracterizados da longevidade, conservado desde leveduras a mamíferos (CHANDRASEKARAN *et al.*, 2017). Os baixos níveis de insulina e IGF-1 induzidos por restrição calórica (CR) ou metformina estão associados a melhora no estado de saúde e ao aumento da longevidade (SOLON-BIET *et al.*, 2015). Curiosamente, os seres humanos com longevidade excepcional apresentam baixos níveis de IGF-1 (MILMAN *et al.*, 2014). Este efeito na expectativa de vida é, pelo menos em parte, devido ao fato do IGF-1 em promover uma via intracelular mediada por PI3K-AKT, permitindo a fosforilação de proteínas conhecidas como *forkhead box O* (FOXO) (WEBB E BRUNET, 2014).

A fosforilação mediada por AKT da FOXO promove a sua exclusão do núcleo para o citoplasma, suprimindo a transcrição dos genes dependentes das proteínas FOXO (WANG *et al.*, 2014). Além disso, a família FOXO é sensível ao potencial redox, sendo as ROS moduladores positivos para sua atividade (KLOTZ *et al.*, 2015). A família FOXO compreende isoformas evolutivamente conservadas (FOXO1, FOXO3, FOXO4 e FOXO6 em mamíferos, DAF-16 em *C. elegans*, DFOXO em *D. melanogaster*) e sua atividade está relacionada a vários processos de defesa celulares incluindo metabolismo da glicose, diferenciação celular, apoptose, reparo do DNA e desintoxicação celular (WANG *et al.*, 2014; MARTINS *et al.*, 2016).

A proteína quinase mTOR (alvo mamífero da rapamicina) é uma serina / treonina quinase atípica que exerce as suas principais funções celulares através da interação com proteínas adaptadoras específicas para formar dois complexos multiproteícos diferentes, mTOR complexo 1 (mTORC1) e mTOR complexo 2 (mTORC2) (SCIARRETTA *et al.*, 2014). O complexo mTOR é um dos principais reguladores celulares da sensibilidade aos nutrientes, sendo ativado na presença de fatores de crescimento e na abundância de nutrientes celulares (LEE *et al.*, 2012). No envelhecimento, a atividade aumentada de mTOR está ligada à deficiência de senescência e autofagia. O tratamento com compostos, tais como a senescência replicativa de atraso da rapamicina, reduz a senescência induzida por danos ao DNA e reduz a disfunção mitocondrial por inibir o complexo mTOR (DAI, KARUNADHARMA, *et al.*, 2014; NACARELLI *et al.*, 2015).

A proteína AMPK é outra reguladora máster para o estado energético celular (BURKEWITZ *et al.*, 2016). Em mamíferos, é ativado quando a relação AMP / ATP e ADP / ATP é elevada, o que ocorre quando a produção de ATP está comprometida. Nestas circunstâncias, a sua resposta tem o propósito de ativar vias catabólicas alternativas de produção de ATP, mais inibindo processos que consomem ATP (HARDIE, 2015; BURKEWITZ *et al.*, 2016). Assim, o AMPK ativa uma série de respostas compensatórias incluindo oxidação de ácidos graxos (β-oxidação), inibição da síntese de ácidos graxos, aumento da biogênese mitocondrial e estimulação da captação de glucose (HARDIE *et al.*, 2012). O tratamento com compostos que aumenta os níveis de AMPK, como a metformina, demonstrou ser benéfico na longevidade, na resistência à insulina e no aumento do desempenho físico (MARTIN-MONTALVO *et al.*, 2013). Além disso, há evidências de que a ativação da AMPK aumenta a expectativa de vida e está relacionada com a melhora do metabolismo em camundongos (RIERA *et al.*, 2016). No entanto, como AMPK atua sobre o envelhecimento é bastante complexo e ainda precisa ser melhor esclarecido.

Sirtuins (SIRTs), o homólogo de regulador silencioso de informação 2 (Sir2) presente em *saccharomyces cerevisiae*, consistem em uma família de proteínas essenciais para mecanismos de defesa celular. Estas proteínas requerem NAD^+ para a sua ativação (IMAI e GUARENTE, 2016). Em mamíferos, existem sete subtipos, localizados em diferentes compartimentos celulares: núcleo (SIRT1, SIRT6 e SIRT7), citosol (SIRT2) e mitocôndrias (SIRT3, SIRT4 e SIRT5) (SRIVASTAVA, 2016). Esta família regula uma série de eventos celulares, incluindo metabolismo, apoptose, fornecimento de energia,

sobrevivência celular, desenvolvimento, diferenciação celular, inflamação e envelhecimento saudável (HAIGIS e SINCLAIR, 2010; HALL *et al.*, 2013). No envelhecimento, SIRT1 estimula a cardioproteção, induzindo resistência ao estresse hipertrófico e oxidativo, também inibe a apoptose dos cardiomiócitos e regula o metabolismo cardíaco (FAVERO *et al.*, 2015). A ativação de SIRT1 induzida por CR melhora a proteção do coração contra isquemia / reperfusão, e este efeito é abolido em camundongos com deleção no gene SIRT1 (YAMAMOTO *et al.*, 2014). Além disso, os compostos que induzem a ativação de SIRT1, como o resveratrol, também parecem induzir a cardioproteção reduzindo a produção de ROS (HSU *et al.*, 2008; FAVERO *et al.*, 2015).

A proteína p53 é conhecida por induzir uma gama de processos anti-proliferativos, tais como a paragem do ciclo celular, conduzindo à senescência e apoptose, em resposta ao stress celular (LIU *et al.*, 2015). Além disso, a p53 desempenha um papel crítico na monitorização e modulação do estado metabólico celular, controlando, pelo menos em parte, processos como a glicólise, a fosforilação oxidativa, a sensibilidade à insulina, a integridade mitocondrial, a oxidação de ácidos graxos e a autofagia (MADDOCKS e VOUSDEN, 2011; RUFINI *et al.*, 2013).

A proteína p53 neutraliza a glicólise por inibição direta da expressão de transportadores de glicose GLUT1 e GLUT4 (SCHWARTZENBERG-BAR-YOSEPH *et al.*, 2004; VOUSDEN e RYAN, 2009), e indiretamente pela inibição de GLUT3 via Nf-kB, resultando numa diminuição na absorção de glucose (KAWAUCHI *et al.*, 2008). Além disso, a p53 controla uma vasta gama de proteínas que participam da glicólise, atuando como um regulador da atividade glicolítica (JIANG *et al.*, 2013; KUNG e MURPHY, 2016). Por outro lado, a p53 promove a fosforilação oxidativa induzindo a expressão da citocromo c oxidase 2 (SCO2) e inibe a piruvato desidrogenase quinase 2 (PDK2) através da parkin (PARK2), regulando a respiração mitocondrial (MATOBA *et al.*, 2006; ZHANG *et al.*, 2011). Assim, a proteína p53 atua conectando o suprimento de energia celular e o estágio senescente, sendo um dos reguladores essenciais para o processo do envelhecimento (BERKERS *et al.*, 2013; KRUISWIJK *et al.*, 2015; WILEY e CAMPISI, 2016).

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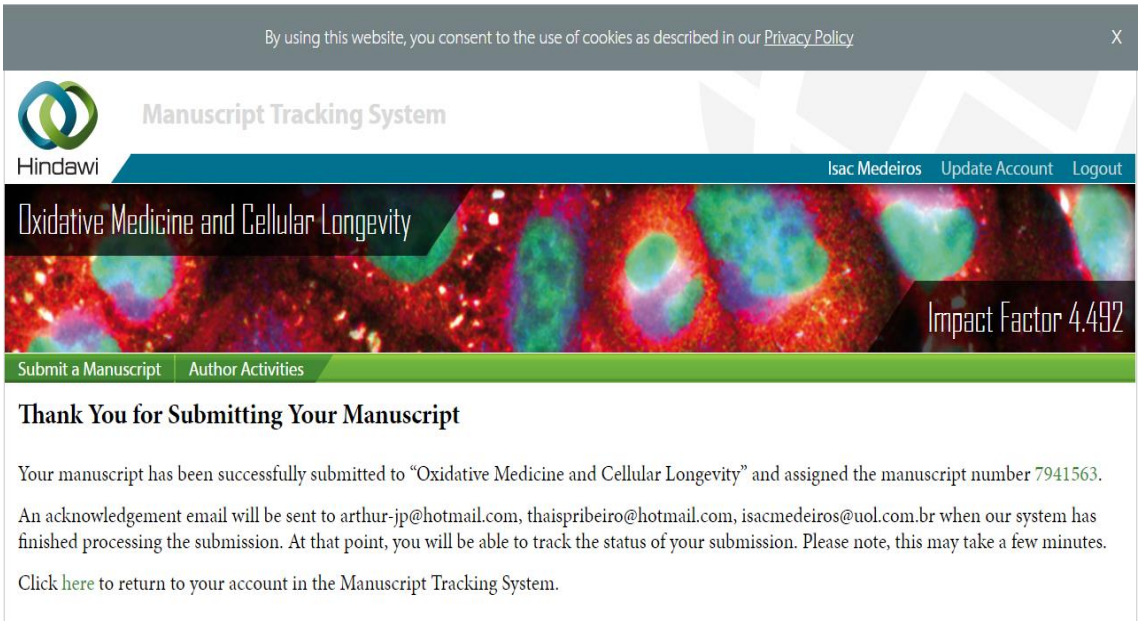

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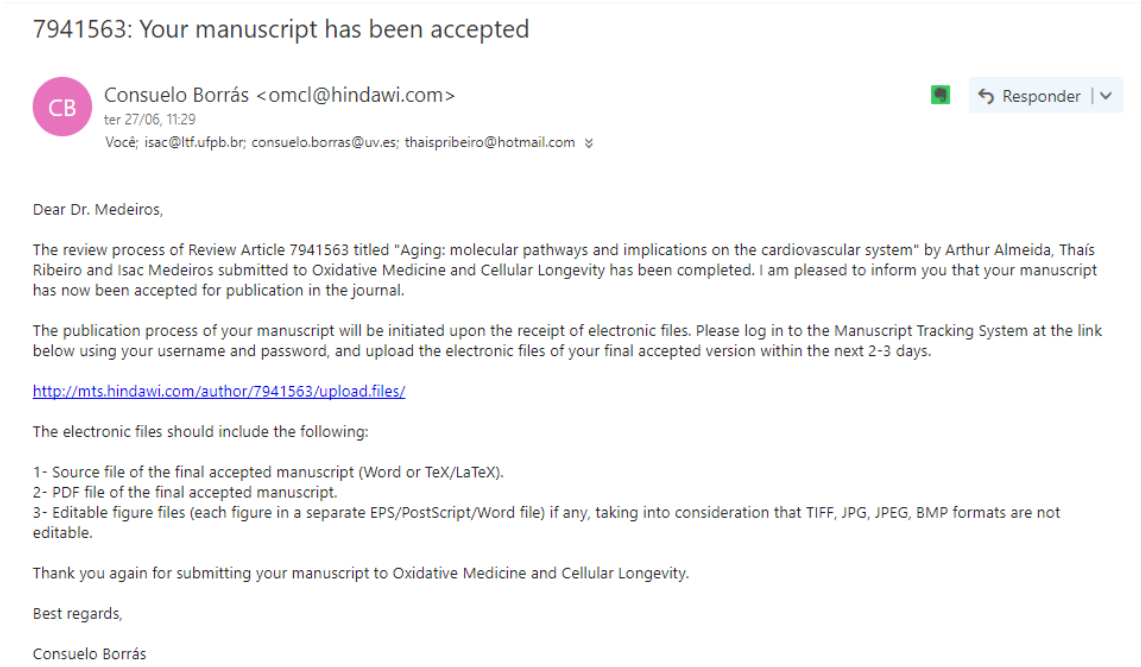

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Responder

Dear Dr. Medeiros,

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Best regards,

Consuelo Borrás

Review Article

Aging: molecular pathways and implications on the cardiovascular system

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Abstract

The world's population over 60 years is growing rapidly, reaching 22% of the global population in the next decades. Despite the increase in the global longevity, individuals' healthspan needs to follow this growth. Several diseases have its prevalence increased by age, such as cardiovascular diseases, the leading cause of morbidity and mortality in the worldwide. Understanding the aging biology mechanisms is fundamental to the pursuit of cardiovascular health. In this way, aging is characterized by a gradual decline in physiological functions, involving the increased number in the senescent cells into the body. Several pathways lead to senescence, including oxidative stress and persistent inflammation, as well as energy failure such as mitochondrial dysfunction and deregulated autophagy, being ROS, AMPK, SIRTs, mTOR, IGF-1 and p53 key regulators of the metabolic control, connecting aging to the pathways who drive to diseases. In addition, senescence can be induced by cellular replication, resulted from telomere shortening. Taken together, it is possible to draw a common pathway unifying aging to cardiovascular diseases, and the central point of this process, senescence, can be the target for new therapies, which may result in the healthspan matching the lifespan.

Keywords: Aging; lifespan; healthspan; senescence; cardiovascular diseases; age-related diseases; DNA damage; oxidative stress; inflammation; mitochondrial dysfunction; autophagy; metabolism; telomeres

1. Introduction

According to the United Nations, the worldwide population over 60 years will grow exponentially over the next decades, rising from 12% in 2015 to 22% in 2050 (Figure 1.A) [1]. Despite the increase of lifespan, individuals not necessarily present an improvement in their quality of life (Figure 1.B). Diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases (CVDs) have its prevalence increased with age, being known as age-related diseases. In 2012, 68% of deaths were associated with these diseases, highlighting to CVDs, corresponding to 46% of this total [2].

Aging is an universal and multifactorial process characterized by a gradual decline of physiological functions, occurring at the molecular, cellular and tissue levels [3], which involve a series of mechanisms such as deregulated autophagy, mitochondrial dysfunction, telomere shortening, oxidative stress, systemic inflammation and metabolism dysfunction [4, 5]. The deregulation of these pathways leads the cell to a senescent state, which contributes to aging phenotype and eventually, driving to age-related diseases (Figure 1.C). Although many theories have been proposed to explain the aging process, neither of them appears to be fully satisfactory.

Therefore, this review draws an integrated approach to aging, addressing the mechanisms that lead the cell to senescence and how this process can contribute to aging and age-related diseases, with emphasis on the cardiovascular system.

2. Senescence - Cellular Retirement

Senescence is the cellular state characterized by cell cycle arrest, usually in G1 phase, but the cells remain metabolically active [6]. Senescent cells secretes a variety of pro-inflammatory cytokines, interleukins and growth factors, which has been reported as “secretory phenotype associated with senescence” (SASP) [7].

Senescent cells are usually removed by the immune system, however, in consequence of immunosenescence, they start to accumulate with age [8, 9]. It is believed that increases in pro-inflammatory mediators are initially a mechanism of “cleaning” the senescent cells, but with immunosenescence, the stimulus generated by the senescent cells are not able to recruit enough functional cells of the immune system, a long-term process that play a negative effect on aging and age-related diseases [10, 11]. Furthermore, there is a limit made by senescence in stem cell lineages “stem cell exhaustion”, resulting in a decreased regenerative potential [12, 13]. These two hallmarks: accumulation of senescent cells and loss in function of regenerative lineages, contribute to aging simultaneously.

Two major pathways control the senescent state: p53/p21 and p16/pRB. Both pathways are complex and have several regulators, but still not totally clarified [3, 14, 15]. In response to DNA-damage (DDR), p53 is stimulated and induces p21 expression, a cyclin-dependent kinase (CDK) inhibitor. In consequence of CDK activity suppression,

the retinoblastoma protein (pRB) is activated. The p16, another CDK inhibitor, also prevents the pRB phosphorylation, leading to pRB inactivation [6, 14, 16]. Thereby, pRB plays a central role in the senescence and its activity is mainly attributed to its ability to bind and inactivate the E2F family of transcription factors, which induces cell cycle proteins and DNA replication factors required for cell growth [16]. In this way, there is a reciprocal regulation between the p53/p21 and p16/pRB signaling, however, these pathways can induce senescence independently [6]. Indeed, "cleaning" naturally occurring p16 positive cells improve healthspan, which present several benefits on the cardiovascular system [17].

Morphologically, the senescent cells are characterized by the increase in volume, and if adherents, they adopt a flattened morphology, however, there is no marker exclusively to senescent state [3]. The first marker to be used was the detection of senescence associated with β-galactosidase (SA-β-gal) activity [18], which actually indicates increased lysosomal activity of β-galactosidase [19]. Recently, several molecular markers were developed and their association with SA-β-gal is the gold standard to confirm the senescent stage. Such markers represent the cell cycle arrest (p16, p21, p53), lack of proliferation markers (Ki67, BrdU), expression of secretion factors (IL-6, IL-8), activation of secretory phenotype regulating pathways (p-p65 or p-p38), changes in chromatin (HP1, Hira) and activation of the DDR (γH2AX, TIFs) (Figure 2) [20, 21].

Several factors leads to senescence, and one of them is the cellular division, with telomere shortening, called replicative senescence [22, 23]. Senescence can also be induced by stress, such as oxidative stress and inflammation leading to DNA damage, activation of oncogenes and changes in chromatin [20]. Another route that leads to senescence is the mitochondrial dysfunction, a process that decreases cellular energy supply, leading the cell to decrease its metabolic activity [24, 25]. In addition, deficiency in the pathways of autophagy also lead the cell to the senescence through the accumulation of cellular "waste", which is toxic to the cell [26].

The raising in the number of cardiac, muscular, endothelial and endothelial progenitor senescent cells has been associated with cardiovascular dysfunction, leading to the progress of several diseases, such as hypertension, atherosclerosis, heart failure and stroke. Therefore, therapies aimed at reversing or delaying the senescence process have been proposed for the treatment of these diseases [17, 27-30].

3. Telomeres - The biological clock

One of the hallmarks of molecular aging is the telomere shortening with the advent of age [4]. Telomeres, known as the biological clock, comprise thousands of nucleotide sequences at the end of each chromosome. In the 3' side, the sequence corresponds to TTAGGG (9-15 kb, in humans) [31]. In somatic cells, after each cell division, part of these bases are lost in the process, promoting telomeres shortening [22]. Thus, it is estimated a finite number of cellular divisions, and after that, cells become senescent (Figure 3) [32].

Associated with telomeres, there is a shelterin complex formed by proteins and transcription factors. This complex comprises a set of six subunits with distinct functions, which has essential participation for chromosome protection [33]. They are telomere repeat-binding factor 1 (TRF1), telomere repeat-binding factor 2 (TRF2), repressor-activator protein 1 (Rap1), TRF1 and TRF2 interacting nuclear protein 2 (TIN2), Tripeptidyl-peptidase 1 (TPP1) and protection of telomere 1 (POT1) [34]. TRF1 and TRF2 bind directly to the double-stranded telomeric repeats, while POT1 recognizes the telomeric strand in the 3' branch. TIN2 binds to TRF1 and TRF2. TIN2 also recruit the TPP1-POT1 heterodimer, reducing different shelterins to organize the final portion of the telomeres. RAP1 is recruited to the telomeres by TRF2. In addition, RAP1 can also bind along chromosomal arms regulating gene transcription [35].

The telomeres participate in the maintenance of the genome and promote stability in the replication process, avoiding undesirable recombination and chromosomal fusion [36, 37]. When the critical telomere size is reached, the proteins cannot be recruited to maintain the T-loop nucleotide sequences. Then the DNA repair system activates cellular checkpoints [38, 39]. Two checkpoints have already been identified that limit cell life in response to telomeres dysfunction: The first checkpoint (M1, the first stage of mortality) is characterized by a complete cell cycle arrest, known as senescence and it is dependent on p53 activation [31]. Cells mutated in the p53 gene may continue to divide even with the critical size of the telomeres reached [33, 40]. If the cell continues to divide, and consequently, the telomeres continue to decrease in size, a new checkpoint is activated (M2, the second stage of mortality), called the crisis. This point is independent of p53 and is characterized by massive chromosomal instability and cell death [41].

In some cellular lineages, such as stem cells, telomeres shortening can be restored by the enzyme telomerase reverse transcriptase (TERT), together with its RNA component (TERC) [42]. Both are regulated by the shelterin complex [43]. The ability of embryogenic or induced pluripotent stem cells (iPSC) to replicate indefinitely is due to a high expression of functional TERT and TERC in these cell populations [44, 45]. Several studies have reported that inducing TERT activity in somatic cells reverses several characteristics of aging, such as senescence [46, 47], which leads to cardioprotection [48]. In addition, hearts expressing TERT showed attenuated cardiac dilation, improved ventricular function and smaller infarct scars concomitant with increased mouse survival by 17% compared with controls [49].

Furthermore, telomeres shortening in circulating lymphocytes, used as an indirect marker of circulating progenitor cells, has been identified as an early-onset alarm for CVDs [50].

Cardiac telomerase activity is detectable at the earliest stages of life and is downregulated in adult rat myocardium [51, 52]. Recently, Richardson and colleagues showed a natural expression of telomerase yet functionally important, in adult mammalian hearts [53], which could be targeted for cardiovascular regeneration.

Therefore, there is a great evidence that combating telomeres shortening has beneficial effects on the cardiovascular system, through slowing or even reversing cellular senescence [49, 54, 55].

4. The role of ROS and oxidative stress - A necessary evil

According to the free radicals theory of aging proposed by Harman in 1956, ROS leads to oxidative damage in cellular biomolecules, contributing to the decline of physiological function with aging [56]. Although a series of reviews and evidences reports the deleterious effects of ROS in aging [57, 58], recent studies on long-lived models and genetically altered animals challenge the role of ROS in aging [59]. In this way, ROS seems to have a double effect. Initially, as an activator of a homeostatic compensatory response that increases with age in order to maintain survival through activation of various defence mechanisms plus stimulating cellular proliferation and, from a certain limit, as a factor that instead of alleviating, aggravates the damages associated with aging (Figure 4.A) [60, 61].

There are several sources of ROS in mammals, including mitochondrial respiration, cyclooxygenase and lipoxygenase, cytochrome p450s, xanthine oxidase, NADPH oxidase, NO synthase, peroxidase, endoplasmic reticulum and other hemoproteins [62, 63].

Many ROS species have unpaired electrons, called free radicals. In this group include superoxide anion (O_2^-•), hydroxyl radical (HO•), nitric oxide (NO•) and lipid radicals. Other reactive oxygen species such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl) are not free radicals but have oxidizing effects that contribute to oxidative stress [64, 65].

The basal balance in ROS levels is mediated by the activity of a set of enzymatic and non-enzymatic complexes with the function of cellular detoxification, collectively called antioxidants [66]. Nuclear factor erythroid 2-related factor 2 (Nrf-2), a transcription factor, is the major regulator of the antioxidant enzymatic system in the vasculature, including transcription of antioxidant enzymes and phase II detoxifiers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), hemeoxygenase-1 (HO-1) and NAD(P)H quinone oxydoredutase-1 (NQO1). Taken together, this system is the major defence system that counteract ROS production *in vivo* [67, 68].

An imbalance to the pro-oxidant side lead to the physiological status known as oxidative stress [69].

NADPH oxidase (Nox) are an important source of ROS on the cardiovascular system [70]. There are seven Nox isoforms: Nox1, Nox2, Nox3, Nox4, Nox5, Duox1 and Duox2. All Nox are transmembrane proteins that have a catalytic site (Nox) and a regulatory protein complex [71]. Isoforms 1, 2, 4 and 5 are expressed in various tissues including the heart and vessels. Nox2 and Nox4 are super-expressed in the vascular tissue of old mice [72]. The prototype of the group is the Nox2 which are composed by 6 subunits: p47phox, p67phox, p40phox and Rac1/2 which are cytosolic regulatory proteins, p22phox which is a membrane regulatory protein, and gp91phox, which is a catalytic subunit present in the membrane [73].

The Nox complex is upregulated by Tnf-α [74] and also by activation of AT1 receptor by angiotensin II [75]. Thus, the increase in ROS production, Nrf-2 begins to have its activity inhibited by the crosstalk with Nf-kB, which is responsible for increasing Tnf-α levels, generating a vicious cycle [76]. As ROS are produced, Tnf-α release increases, aggravating oxidative stress. This shift in the expression of Nrf-2 to Nf-kB seems to be gradual, accompanying aging, and directly proportional to the incidence of CVDs (Figure 4.B).

The main source of ROS during aging is the mitochondria [77]. Harman in 1972, reviewed his theory about free radicals after the discovery that mitochondria turned oxygen into water, a process that, when deficient, result in a high production of superoxide anions, raising mitochondrial ROS (mtROS) levels, which lead to the accumulation of mitochondrial DNA (mtDNA) mutations, driving to mitochondrial dysfunction, resulting in aging [78]. However, recent evidences involving mtROS using longevity animals modelling rejects, at least in part, the original idea of the mitochondrial theory of aging [61]. These pathways conserved from yeast to mammals have been subsequently assessed for their role in regulating longevity, as well as to begin assessing their roles in CVDs [79].

Studies with *C. elegans* report that, by deletion of the SOD2 gene, the increase in mtROS seems to prolong lifespan [80]. In *Drosophila*, mtROS from the electron transport chain also appears to have a positive effect on the lifespan [81]. In addition, overexpression of catalase increase resistance to oxidative stress but do not improve lifespan [82]. In worms, antioxidant diets reduce their lifespan [83]. In mice, genetic alterations that increase mtROS and oxidative damage do not accelerate aging, although induce the appearance of various age-related diseases [84]. There is evidence that mtROS and cytosolic ROS have opposite effects, being the cytosolic more toxic to the cell [85].

Therefore, the H_2O_2 produced with beneficial propose in the mitochondria, eventually diffuse through the mitochondrial membrane [86], reaching to the cytoplasm contributing to the oxidative stress involved in aging, suggesting that ROS effects are dependent on where it is present and its concentration [85].

The elderly are more susceptible to oxidative stress due to a reduction in the efficiency of their endogenous antioxidant systems. Organs such as heart, which it has a limiting rate of replication and high levels of oxygen consumption, are particularly sensitive to this phenomenon, which explains, in part, a high prevalence of CVDs in aging [87]. On the other hand, in endothelial cells, ROS derived from NADPH oxidase complex induces *in vivo* kinases pro-survival via AMPK, plus an additional effect of inducing autophagy, improving the vascular function in aged mice coronary [88]. Thus, this approach can integrate paradoxical concepts about the beneficial, deleterious or neutral role of ROS in aging.

5. Inflammation - A war without an army

Aging is accompanied by a systemic increase of pro-inflammatory agents, a phenomenon known as "inflammaging" [89]. Senescent cells have the ability to release pro-inflammatory agents (ASAP) capable of attracting defence cells, that phagocytosis the senescent cells [90, 91]. However, in aging, the exhaustion of stem cells occurs, reducing the regenerative capacity of the organism, as well as the production of functional immunity cells, a term known as immunosenescence, allowing the accumulation of senescent cells into the body, which is related to the onset of cardiovascular diseases (Figure 8.B) [8, 92].

The ASAPs components include agents such as Tumor Necrosis Factor-α (Tnf-α), Interleukin-6 (IL-6) and IL-1β [93]. These pro-inflammatory agents are mainly regulated by transcription factors sensitive to redox potential, as the activator of protein-1 (AP-1) and nuclear factor kappa B (Nf-kB) [94]. Overproduction of ROS is essential for activating AP-1 and Nf-kB through the stress of kinases such as extracellular signal regulatory kinases (ERKs), c-jun N-terminal kinases (JNKs), p38 activated protein kinase Mitogen (p38 MAPK), protein kinase C (PKC), phosphatidylinositol-4,5 bisphosphate 3-kinase (PI3K), Akt and Src family kinases (SFK) [95].

This leads to increased expression of inflammatory target proteins such as matrix metalloproteinase-9 (MMP9), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), cytosolic phospholipase A2 (cPLA2) and pro-inflammatory mediators such as the TNF-α, IL-1 and IL-6. Many of these inflammatory proteins or their products such as iNOS, COX, and PGE2 are prominent sources of ROS [95-98]. In fact, the presence of these inflammatory biomarkers are related to the endothelial damage, VSMCs proliferation and matrix remodelling, being associated to the genesis and progression of atherosclerosis and hypertension even in the absence of traditional risk factors such as smoking and obesity [99-101].

Moreover, targeting the overexpression of Nf-kB by anti-inflammatory molecules seems to play positive effects on the prevention of cardiovascular diseases by slowing the vascular remodelling [102].

Therefore, one of the fundamental features associated with cardiovascular aging is the crosstalk between oxidative stress and inflammation (Figure 4.B). It is necessary to point out that both processes contribute to the physiological organism defence, and in the young individual, these processes are with their basal functional activity. For an activation of the inflammatory signaling, it is necessary an increase in the redox potential, which is achieved by stimulating ROS generation, such as that mediated by the mitochondria.

6. Mitochondrial dysfunction - Communication failure

Mitochondria are considered the cellular “powerhouse”, since they have the ability to generate adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS), providing chemical energy for cellular survival and function [24]. In addition, there is

evidence that mitochondria play a non-energetic role in the regulation of metabolism, apoptosis, innate immunity and cardiovascular aging [103-105].

Despite most mitochondrial genes were transferred to the nuclear genome, 13 subunits essential for OXPHOS activity remain encoded by mtDNA. The other 76 subunits are encoded by nuclear genome, being synthetized in the cytoplasm and imported to the mitochondria, requiring functional communication between both genomes [106, 107]. This functional interaction is essential for mitochondrial health and the failure of this communication leads to mitochondrial dysfunction, decreasing ATP synthesis [105]. In this way, the failure in energy status drives to endothelial dysfunction, plus inflammation and oxidative stress, being related to vascular remodelling [108]. In addition, mitochondrial dysfunction is associated to heart failure, leading to a deregulation of the cardiovascular system [109].

The mitochondria regulation occurs mainly by peroxisome proliferator-activated receptor-γ coactivators α and β (PGC-1α and PGC-1β), which responds to changes in nutrient status, such as the ratio of NAD^+/NADH and AMP/ATP (regulated through SIRT1 and AMPK, respectively) [110, 111]. The expression of PGC-1α/β play a fundamental role in mitochondrial biogenesis, promoting protection to the endothelium, and consequently promoting cardioprotection [112, 113].

Recently, Gomes and colleagues described a process of mitochondria regulation via HIF-1α, independent of PGC-1α/β, in response to SIRT1 activity, which it is controlled by nuclear NAD^+ levels. Six hours after induction of deletion of the SIRT1 gene in myoblasts, HIF-1α levels begin to rise, and after 12 hours loss of mitochondrial homeostasis occurs, although ROS levels only increase after 24 hours of the procedure [114]. This HIF-α-mediated ROS in *C. elegans* is the main determinant of lifespan, but the mechanisms involved are still not fully understood [115].

In aging, there is no loss in SIRT1 levels in the body, but NAD^+ levels decrease with age, leading to a downregulation of SIRT1 activity [116]. As a result, a pseudo-hypoxic state occurs, decreasing the activity of complexes I, III, IV and V (encoded by nuclear and mitochondrial genomes) but not complex II (encoded by the nuclear genome) [106]. To recover the activity of complexes I, III, IV and V, it is necessary to restore mtDNA and nuclear DNA communication, which is achieved by NMN supplementation (NAD^+ precursor). Treatments that restore NAD^+ levels have been shown to be beneficial in restoring mitochondrial function and several aspects related to aging in mice [114, 116, 117], indicating that aging is, at least in part, caused by a failure in nuclear-mitochondrial communication. A process that is dependent of energetic cellular balance [111, 114].

Treatments that promote mitochondrial health, drives to an improvement in metabolism and heath aging, being related to several benefits on the cardiovascular system [118-120]. In addition, targeting mitochondria seems to have positive effects on the treatment of heart failure [121].

In a wide perspective, it is possible to identify that a failure in the cellular energy create a stressful environment that eventually leads to senescence (Figure 5). The ROS

increased with the mission of stimulating survival mechanisms, also promote DNA damage, driving to aging. It is still not clear the relation between ROS and NAD^+ levels, which could help to understand the increased redox potential in the cell. Moreover, to maintain the energy status in satisfactory levels, mechanisms that counteracts ATP depletion such as autophagy play fundamental role, protecting cells from the energy failure due to mitochondrial dysfunction.

7. Autophagy - Cellular scavengers

Autophagy or "self-eating" refers to the lysosomal degradation process that removes protein aggregates, damaged organelles, toxic substances and even pathogens [122]. This process is essential to maintain cell integrity and homeostasis by providing metabolites for cell survival under stress conditions [123]. In addition, it helps to maintain cellular energy levels during nutrient limitations through catabolic recycling processes [124].

There are three types of autophagy currently described, namely: Macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). All differ in their mechanisms and functions [125]. Microautophagy produces random invaginations in the lysosomal membrane, involving nearby cytoplasmic components to the lysosomal lumen [126]. CMA acts directly through the lysosomal membrane via the specific receptor, LAMP-2A (lysosomal associated membrane with protein type 2) [127]. Macroautophagy, often referred as autophagy, requires formation of a double membrane (autophagosome) involving the material to be degraded and subsequently being fused to the lysosome (Figure 6) [128].

Autophagy is mainly downregulated by the mTOR complex. This complex is activated under nutrient-rich conditions, playing a fundamental role in the nutrient sensitivity [129]. In a nutrient-poor condition, another energetic sensor is activated, AMPK, which directly inhibits mTOR by its direct phosphorylation as well as directly activates the ULK1 (ATG protein family) (ATG -genes related to autophagy), stimulating autophagy [128]. The nutrient shortage also regulates the autophagy at the transcriptional level by modulating the expression of ATG encoded genes, and this mechanism is mediated, at least in part, by the transcription factor FOXO1 [130].

In aging, autophagy is deregulated or inoperative, favoring the accumulation of "garbage" into the cell [131]. Overexpression of mTOR complex during aging increases abnormal proteins aggregates, being related to the genesis of CVDs [132]. On the other hand, enhancing autophagy by mTOR inhibition or AMPK activation, leads to an increase in healthspan, improving the cardiovascular function and prevents CVDs [133, 134]. However, its excessive autophagy activation seems to have deleterious effect on the cardiovascular system [135]. Thereby, autophagy seems to be a compensatory effect on cellular energy levels, that depend of mitochondrial dysfunction, and understand the crosstalk between both regulators are essential to connect the energetic signalling to metabolism.

8. Metabolic control of aging – Connecting the dots

Aging is characterized by a decrease in cellular energy supply [7]. The major regulators of this process are the mitochondria as a source of ATP and the lysosomes, an essential organelle for the autophagy, one of the mechanisms responsible for generating energy in times of nutrient scarcity [136, 137]. Several mechanisms that enhance the function of these processes play a beneficial role in lifespan and healthspan [131, 138]. The mechanism involved in this process has several regulators such as Insulin/IGF-1, mTOR, AMPK, and Sirtuins [139]. Other factors such as ROS and p53 pathway also appear to be part of cellular energy control (Figure 8) [140, 141].

The insulin/growth-factor-1 (IGF-1) pathway controls survival, proliferation and metabolic processes. This mechanism is one of the well-characterized pathways of lifespan, conserved from yeast to mammals [142]. Low levels of insulin and IGF-1 induced by caloric restriction (CR) or Metformin are associated with improved healthspan and increased longevity [139]. Interestingly, humans with exceptional longevity present low IGF-1 [143]. This effect on lifespan is, at least in part, due to the fact that IGF-1 promotes an intracellular pathway mediated by PI3K-AKT, allowing the phosphorylation of proteins known as Forkhead box O (FOXO) [144].

The AKT-mediated phosphorylation of FOXO promotes its exclusion from the nucleus to the cytoplasm, suppressing gene transcription dependent on FOXO proteins [145]. In addition, the FOXO family is sensitive to the redox potential, being ROS levels positive modulators for their activity [146]. The FOXO family comprises evolutionarily conserved isoforms (FOXO1, FOXO3, FOXO4 and FOXO6 in mammals, DAF-16 in *C. elegans*, DFOXO in *D. melanogaster*) and its activity is related to various cellular processes including glucose metabolism, cell differentiation, apoptosis, DNA repair and cellular detoxification [145, 147].

The protein kinase mTOR (mammalian target of rapamycin) is an atypical serine/threonine kinase that exerts its main cellular functions by interacting with specific adaptor proteins to form two different multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [148]. The mTOR complex is one of the major cellular regulators of nutrient sensitivity, being activated in the presence of growth factors and in abundances of cellular nutrients [129]. In aging, increased mTOR activity is linked to senescence and autophagy deficiency. Treatment with compounds, such as rapamycin delay replicative senescence, reduces senescence induced by DNA damage and reduces mitochondrial dysfunction by inhibit the mTOR complex [149, 150].

AMPK is another master regulator to cellular energy status [151]. In mammals, it is activated when AMP/ATP ratio and ADP/ATP is elevated, which occurs when ATP production is compromised. Under this circumstances, its response has the purpose to activate alternative catabolic ATP-producing pathways, plus by inhibiting ATP-consuming processes [151, 152]. Thus, AMPK activates a series of compensatory responses including fatty acid oxidation (β-oxidation), inhibition of fatty acid synthesis, increased mitochondrial biogenesis and stimulation of glucose uptake [153]. Treatment with compounds that increases AMPK levels, such as metformin, has been shown to be beneficial in longevity, insulin resistance and increase in physical performance [154]. In

addition, there is evidence that AMPK activation increases the lifespan and is related to the improvement of metabolism in mice [5]. However, how AMPK acts on aging is quite complex and still remains to be clarified.

Sirtuins (SIRTs), the homologue of Silent Information Regulator 2 (Sir2) present in *Saccharomyces cerevisiae*, consist of a family of essential proteins for mechanisms of cell defence. These proteins require NAD^+ for its activation [155]. In mammals, there are seven subtypes, located in different cellular compartments: nucleus (SIRT1, SIRT6, and SIRT7), cytosol (SIRT2) and mitochondria (SIRT3, SIRT4 and SIRT5) [156]. This family regulates a range of cellular events including metabolism, apoptosis, energy supply, cell survival, development, cellular differentiation, inflammation and healthy aging [157, 158]. In aging, SIRT1 stimulate cardioprotection, inducing resistance against hypertrophic and oxidative stress, also inhibits cardiomyocytes apoptosis, and regulates cardiac metabolism [159]. SIRT1 activation induced by CR improves heart protection from ischemia/reperfusion, and this effect is abolished in SIRT1 knockout mice [160]. In addition, compounds that are able to induce SIRT1 activation, such as resveratrol, also appears to induce cardioprotection by reducing ROS production [159, 161].

The p53 protein is known to induce a range of anti-proliferative processes, such as cell cycle arrest, leading to senescence and apoptosis in response to cellular stress [162]. In addition, p53 plays a critical role in monitoring and modulating cellular metabolic status, controlling, at least in part, processes such as glycolysis, oxidative phosphorylation, insulin sensitivity, mitochondrial integrity, fatty acid oxidation and autophagy [163, 164].

p53 counteracts glycolysis by directly inhibiting the expression of GLUT1 and GLUT4 glucose transporters [165, 166], and indirectly by inhibiting GLUT3 via Nf-kB, resulting in a decrease in glucose uptake [167]. In addition, p53 control a wide range of proteins that participate in glycolysis, acting as a glycolytic activity regulator [168, 169]. On the other hand, p53 promotes oxidative phosphorylation by inducing the expression of cytochrome c oxidase 2 (SCO2) and inhibits pyruvate dehydrogenase kinase 2 (PDK2) through Parkin (PARK2), regulating mitochondrial respiration [170, 171]. Thus, p53 protein acts by connecting the cellular energy supply and senescent stage, being one of the most important regulators for the aging process [140, 172, 173]. The same mechanisms that leads to aging described above are implicated on the cardiovascular system, being related to the balance between health to diseases, including the CVDs.

9. Aging - Implications on the cardiovascular system

Cardiovascular aging is defined as an age-dependent progressive degeneration, which makes the heart and vessels more vulnerable to stress, contributing to increased mortality and morbidity [174]. Notably, the vascular aging is characterized by molecular, structural, cellular and physiological changes, being aging the main risk factor in the pathogenesis of CVDs [175, 176].

In the aged heart, several complex modifications including diastolic dysfunction, left ventricular hypertrophy, increased risk of atrial fibrillation, valvular degeneration, leads to a decreased maximal exercise capacity, which are related to heart failure [177].

Under normal conditions, vessels have the ability to respond to various stimuli, such as vasoconstriction due to an adrenergic or circulatory (e.g. Angiotensin II or Endothelin II) agonist response [178]. On the other hand, vasodilator mediators such as nitric oxide (NO), *endothelium-derived hyperpolarizing factor* (EDHF) and some prostaglandins (e.g. PGI_2) have the mission of balancing the vascular tonus [179, 180].

In particularly, the production of NO is the major marker of the vascular function [181, 182]. In the vessel, its synthesis is made mainly by endothelial nitric oxide synthase (eNOS), being aging associated to a decrease in the NO production [183-185]. In senescent-accelerated mice, endothelial dysfunction associated with aortic aged is linked to eNOS dysfunction [186]. Increased release of ROS and subsequent inactivation of NO is an important mechanism involved on the impairment of endothelium-dependent vessel relaxation, leading to stiffness and vascular inflammation [187, 188].

The vascular aging leads to thickening of the intima and media layer (vascular remodeling), as well as gradual loss of arterial elasticity, resulting in vascular rigidity [189, 190]. Increased collagen and decreased elastin content, promoted at least in part by age, in addition to increased glycosylated proteins, matrix metalloproteinase activity and systemic stimuli such as angiotensin II signaling, are linked to vascular rigidity [191, 192]. Aged endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) also show increased secretion of pro-inflammatory cytokines, derived in large part by senescent cells, which results in persistent vascular inflammation [29, 100]. In addition, VSMCs change its metabolic route to promote aerobic glycolysis (in response to mitochondrial dysfunction), which is essential to produce high rate of substrate for cellular growth and proliferation, and express factors such as *vascular endothelial growth factor* (VEGF), platelet-derived growth factor (PDGF-β) and transforming growth factor alpha (TGF-α) that contribute to the vascular remodelling (Figure 8 A) [193, 194].

The vessels also play an important role in connect all the tissues through the blood flow. In fact, the vascular inflammation extends to other organism components leading to a systemic effect [195]. In the young blood, there is a predominance of growth factors in detriment of inflammatory mediators, plus healthy immunity cells and endothelial progenitor cells, essential for vascular "cleaning" and regeneration, respectively [196]. On the other hand, the aged blood have predominance in pro-inflammatory factors, released largely by senescent cells [197]. In addition, there is a failure of immune system, resulting in the acumulation of senescent cells in the tissue, leading to an stressfull enviroment, which are associated to the development and progression of CVDs [195, 198].

Changing the systemic influence from the blood by connecting young to aged blood by parabiosis (surgical technique that unites the vasculature of two living animals), showed that after 4 weeks, aged rats that was exposed to young circulation had reversed

age-related cardiac hypertrophy, leading to an improvement on the cardiovascular system [199].

Thus, the vascular remodeling, by aging or pathological conditions, is accompanied by oxidative stress and inflammation, leading to an increase of senescent cells in these tissues (Figure 8.B) [58, 200]. The endothelial cells have fundamental importance in the development of vascular remodeling, being endothelial dysfunction target of therapies against CVDs, such as hypertension, atherosclerosis, and heart failure [201-203]. Treat aging, seems to show several benefits on the cardiovascular system, by creating a healthy systemic environment, which slow the progression of endothelial dysfunction and the vascular remodeling associated with aging, leading to cardiovascular protection.

10. Conclusion and future directions

In this review, we discuss cellular mechanisms related to aging. It is possible to notice that aging is a multifactorial process that encompasses intrinsic factors to several species and the accumulation of senescent cells is common in the main part of them. Understanding the aging process, we may find the genesis of age-related diseases, since many of them are characterized by disorders that are consequences of cellular dysfunction caused by senescence. This accumulation of senescent cells can have a replicative genesis, bringing into action therapeutic targets such as telomerase, as well as induced by stress, such as the cellular energetic loss, which encompasses the mitochondria dysfunction and deregulated autophagy. These mechanisms are connected by a series of proteins, transcription factors and environmental factors into the cell, such as redox potential. However, a determinant factor controlling the whole process remains unclear. One of the candidates would be to understand how the redox potential determines gene expression and promotes responses in metabolism. The fact that ROS promotes an increase in redox potential. and this hallmark is involved in aging as well as in age-related diseases, makes us to believe that the increase in cellular ROS is intentional by the cells, in order to promote cellular survival mechanisms, requiring more and more ROS to have the same effect over time, a process that drives to the deleterious effects of ROS. Understanding how the concentration and localization of ROS and its interaction with longevity genes may be a key point to understand the complex metabolic mechanism that controls aging. In this way, it will be possible, in the future, to take a pill that promotes an increase in longevity and, in addition, play a role in minimizing the onset of aging-related diseases.

Conflict of interests

The authors report no conflict of interests. The authors are responsible for the content and writing of this paper.

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Author Contributions

The three authors equally contributed to the composition of the manuscript.

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Figure Legends

Figure 1. Aging and health. A) The global population will increase from 12% in 2015 to almost 22% in 2050 [1]. **B)** Despite the increase in lifespan, the individual healthspan does not follow this growth, which means that targeting aging with new therapies are essential to minimize the onset of aging-related diseases. **C)** At the cellular level, aging is characterized by an increase of senescent cells in the organism, caused by several factors, including oxidative stress, systemic inflammation, mitochondrial dysfunction, deregulated nutrient sensitivity, autophagy dysfunction, and telomere shortening. The same mechanisms that lead to aging, drives to age-related diseases, in particular, the cardiovascular diseases, the major cause of death in the worldwide.

Figure 2. Senescence and aging. Aging is characterized by senescent cells accumulation into the body. Senescence can be achivied replicativelly, or induced by stress. Once activated, the p16 and p53/p21 pathways converge with each other, regulating the Rb mechanism, leading to cell cycle arrest, and consequently, the senescence. This results in the release of cytokines and chemokines, driving to a systemic inflammatory condition that lead to aging and age-related diseases. The senescent cells are characterized by a high lysosomal β-galactosidase activity, and in association with others characteristic factors, consist the gold standard for the senescence characterization.

Figure 3. Role and function of telomeres in DNA protection. After each cell division, each chromosome loses a part of its telomeres, a region characterized by thousands of repeated sequences of nitrogenous bases. At a critical point, cells with shortened telomeres stop to divide, leading to senescence and resulting in aging and CVDs. Cells with high replicative rates, such as Stem cells lineages express telomerase, an enzyme capable of reversing telomere shortening. This enzyme plays a key role in the development of new therapies that aim to slow or reverse the aging process.

Figure 4. Potential redox controls cells fate. One of the hallmarks of aging is the increase in ROS levels production. New approaches define this increase as a compensatory cellular response with the original purpose to maintain cellular homeostasis, and from a certain limit, as a factor that aggravates aging. **A)** The increase in ROS levels, first as a factor that activates survival pathways, continues to increase as a consequence of the deficiency in the antioxidant system, generating other cellular responses such as apoptosis, and with a failure in apoptotic signaling, driving to severe cellular damage, such as necrosis. **B)** Several sources of ROS

contribute to the increase of redox potential, a factor that shifts the balance to the transcription of pro-inflammatory factors, while the antioxidant genes are silenced, connecting ROS and inflammation to aging.

Figure 5. Schematic overview of nucleus-mitochondria communication in aging. Decreased SIRT1 activity due to decreased NAD^+ levels leads to changes in various genes expression. 1) Decreased PGC-1α / β levels, leading to decline the mitochondria biogenesis. 2) Increased expression of HIF-α, leading to a pseudo-hypoxia state, and consequently, a miss nucleus-mitochondria communication, driving to a failure in coding OXPHOS genes. 3) Increased expression of Nf-kB levels, leading to inflammation, plus decreasing NAMPT production, a precursor of NAD^+. 4) Decreased expression of FOXO, a factor that participates in cytoprotection. These responses are accompanied by increased ROS levels, a factor that activates AMPK, acting together as factors that counteract the decrease of SIRT1 activity. Increasing the ROS levels from a certain limit, promote DNA damage, creating a paradoxical effect. In the mitochondria, the failure in the OXPHOS leads to a decrease in energy supply, and combined with oxidative stress, drive to mitochondrial dysfunction. These factors combined lead to cellular stress and consequently to senescence. The interaction of oxidative stress and NAD^+ levels are still unclear and may be an important source to understand how redox potential controls cellular energy metabolism.

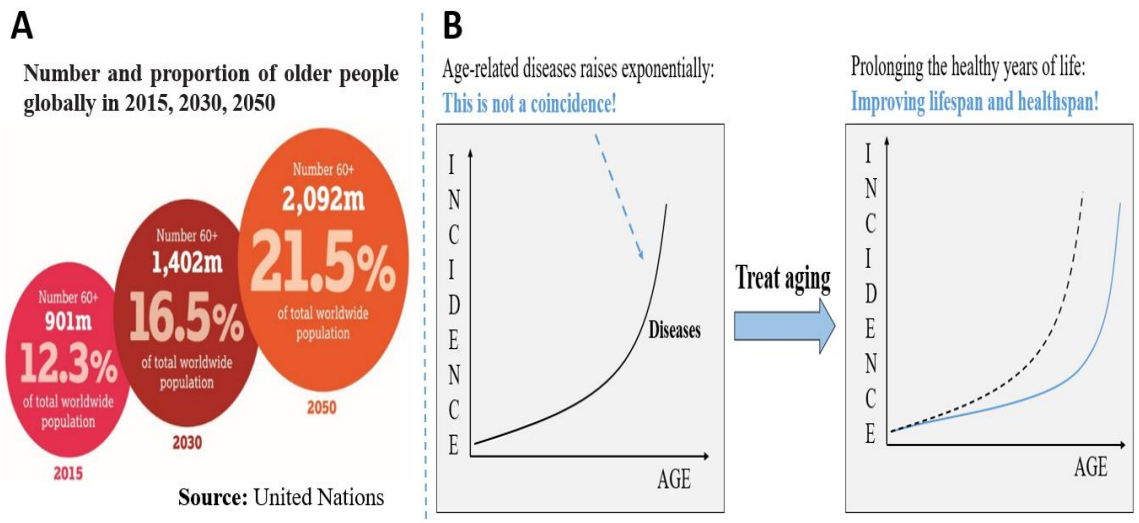
Figure 6. Role of autophagy as cellular scavengers. Autophagy is mainly regulated by two energy sensors: mTOR and AMPK. mTOR is an inhibitor of autophagy and is activated when there are abundant cellular nutrients. AMPK is activated when nutrients deplete, inducing autophagy by inhibiting mTOR, as well as direct activation of autophagy. This mechanism is important for cell “cleaning”, degrading damaged organelles, protein aggregates, and other cellular-toxic components. After the formation of the autophagosome, there is fusion with the lysosome, occurring the cleavage of the degraded material. There are two other types of autophagy: microautophagy, with direct involvement of the material by the lysosome. In addition, there is a chaperone-mediated autophagy, encompassing the material via the LAMP-2 receptor. Together, these mechanisms improve metabolism, being an energy source through recycling amino acids, and eventually, participating in cellular quality control, which promotes an improvement in the individual lifespan and healthspan.

Figure 7. Metabolic control involved in aging and on the cardiovascular system. IGF-1, mTOR, AMPK, SIRT1, p53 and ROS are key regulators in metabolic control. Many of these pathways are complex involving crosstalk between them, with many paradoxical effects. The stimulation of the IGF-1 pathway by insulin promotes PI3K/AKT pathway activation, which induce the exclusion of FOXO from the nucleus, inhibiting its function, in addition, IGF-1 activate eNOS, increasing NO availability, improving the vascular function. IGF-1 also activates the RAS/p38MAPK pathway inducing mechanisms of cell growth and proliferation. Finally, IGF-

1 stimulates vesicles containing GLUT-4 to the cell membrane, promoting the uptake of glucose, the main cellular energy substrate. There are also other two glucose transporters that help in glucose uptake such as GLUT-1 and GLUT-3, the last one can be downregulated by p53 via Nf-kB. Under normal conditions, most pyruvate is directed to the mitochondria, producing ATP by OXPHOS. In age, NAD^+ levels decrease, driving to a loss in SIRT1 activity, resulting in mitochondrial dysfunction via PGC-1α / β and HIF-α. Thus, pyruvate is directed to lactate production, even in the presence of O_2, a process known as "The Warburg effect". This metabolic shift is essential for increasing biomass, stimulating cell growth, proliferation and differentiation, which promote angiogenesis. In this way, a mitochondrial dysfunction result in a decreased ATP production, activating AMPK. This protein stimulates autophagy, generating energy for the cell. In addition, it stimulates p53, which inhibit the uptake and conversion of glucose, plus to stimulate OXPHOS activity, generating an anti-proliferative effect. Finally, ROS produced by mitochondrial dysfunction stimulates several signaling pathways, such as AMPK, but also activates AKT, which stimulates mTOR, being the redox potential the major regulator of this balance.

Figure 8. Young and aged vascular comparison in two different perspectives. In vascular aging, the remodeling occurs due to the accumulation of senescent and dysfunctional cells in response to the environmental changes caused by age. **A)** In the aged vessel, there is a loss of the vessel elasticity, due to the raises of contracting factors, plus an increase in the number of muscle cells. These factors combined drive to the matrix change, with inflammatory infiltrates and fibrosis, leading to vascular hypertrophy. **B)** In young blood, there is a predominance of growth factors, in addition to healthy cells of immunity and progenitor cells driving to vascular “cleaning” and regeneration, respectively. In the aged blood, it is checked a predominance in pro-inflammatory factors, released largely by senescent cells. The senescent cells accumulate with age in response to a failure of the immune system, a term known as immunosenescence. In addition, there is an increase in fibroblast proliferation, leading to an stressfull enviroment related to the vascular remodelling.

Figure 1


A
Number and proportion of older people globally in 2015, 2030, 2050
Number 60+
901m
12.3%
of total worldwide population
2015
Number 60+
1,402m
16.5%
of total worldwide population
2030
Number 60+
2,092m
21.5%
of total worldwide population
2050
Source: United Nations
B
Age-related diseases raises exponentially:
This is not a coincidence!
INCIDENCE
Diseases
AGE
Treat aging
Prolonging the healthy years of life:
Improving lifespan and healthspan!
INCIDENCE
AGE

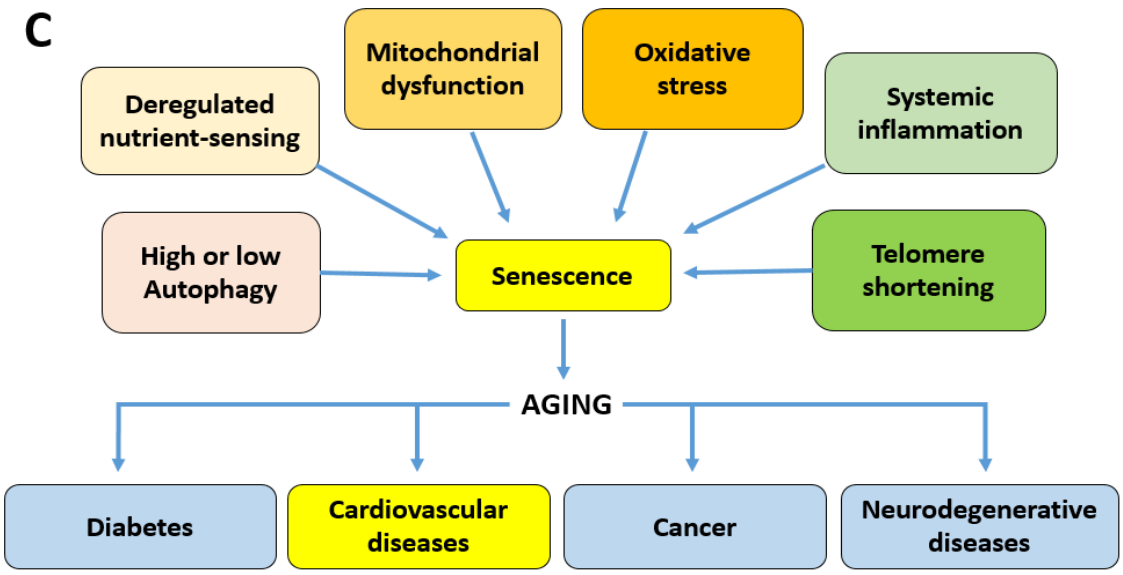

C
Deregulated nutrient-sensing
Mitochondrial dysfunction
Oxidative stress
Systemic inflammation
High or low Autophagy
Senescence
Telomere shortening
AGING
Diabetes
Cardiovascular diseases
Cancer
Neurodegenerative diseases

Figure 2

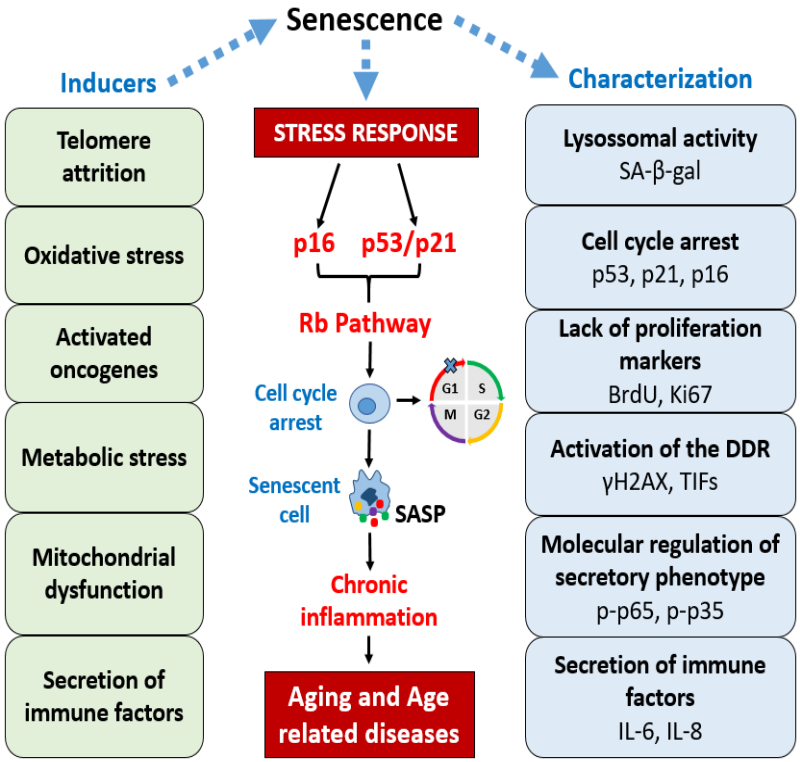

Senescence
Inducers
Characterization
Telomere attrition
Oxidative stress
Activated oncogenes
Metabolic stress
Mitochondrial dysfunction
Secretion of immune factors
STRESS RESPONSE
p16
p53/p21
Rb Pathway
Cell cycle arrest
G1
S
M
G2
Senescent cell
SASP
Chronic inflammation
Aging and Age related diseases
Lysossomal activity
SA-β-gal
Cell cycle arrest
p53, p21, p16
Lack of proliferation markers
BrdU, Ki67
Activation of the DDR
γH2AX, TIFs
Molecular regulation of secretory phenotype
p-p65, p-p35
Secretion of immune factors
IL-6, IL-8

Figure 3

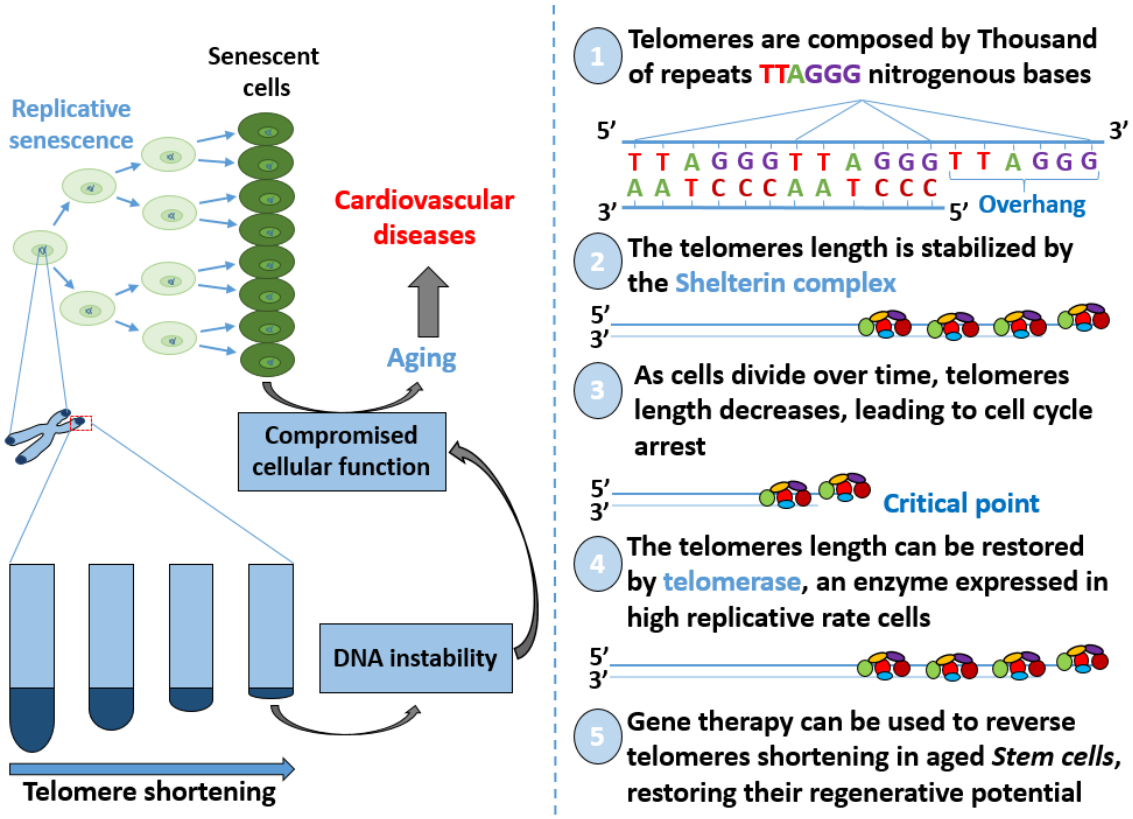
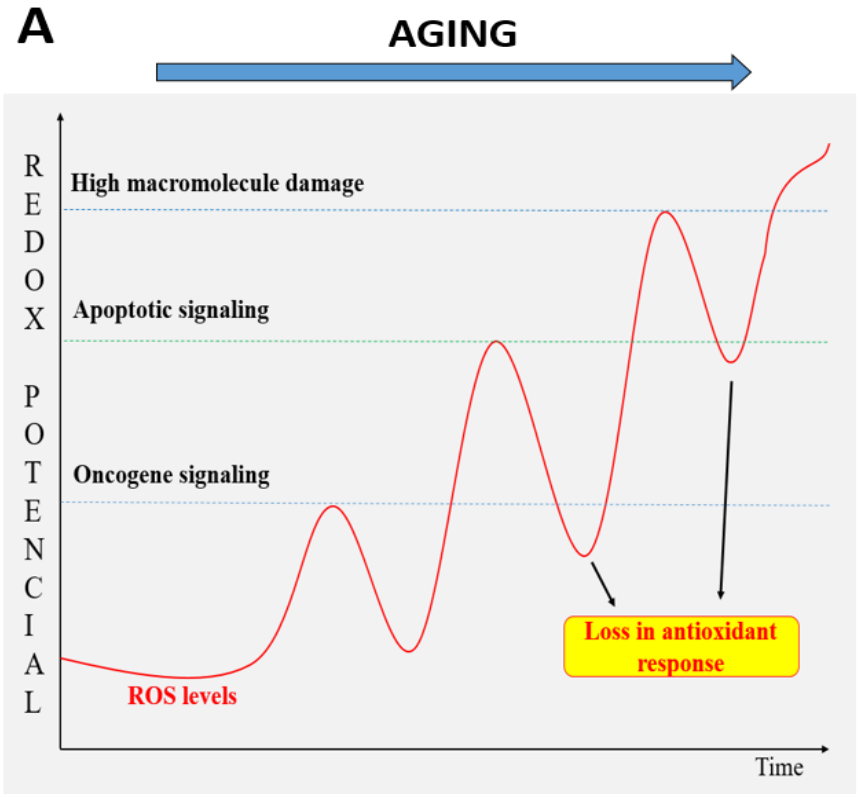

Senescent cells
Replicative senescence
Cardiovascular diseases
Aging
Compromised cellular function
DNA instability
Telomere shortening
1 Telomeres are composed by Thousand of repeats TTAGGG nitrogenous bases
5′
3′
T T A G G G T T A G G G T T A G G G
A A T C C C A A T C C C
3′
5′ Overhang
2 The telomeres length is stabilized by the Shelterin complex
5′
3′
3 As cells divide over time, telomeres length decreases, leading to cell cycle arrest
5′
3′
Critical point
4 The telomeres length can be restored by telomerase, an enzyme expressed in high replicative rate cells
5′
3′
5 Gene therapy can be used to reverse telomeres shortening in aged Stem cells, restoring their regenerative potential

Figure 4


A
AGING
REDOX POTENCIAL
High macromolecule damage
Apoptotic signaling
Oncogene signaling
ROS levels
Loss in antioxidant response
Time

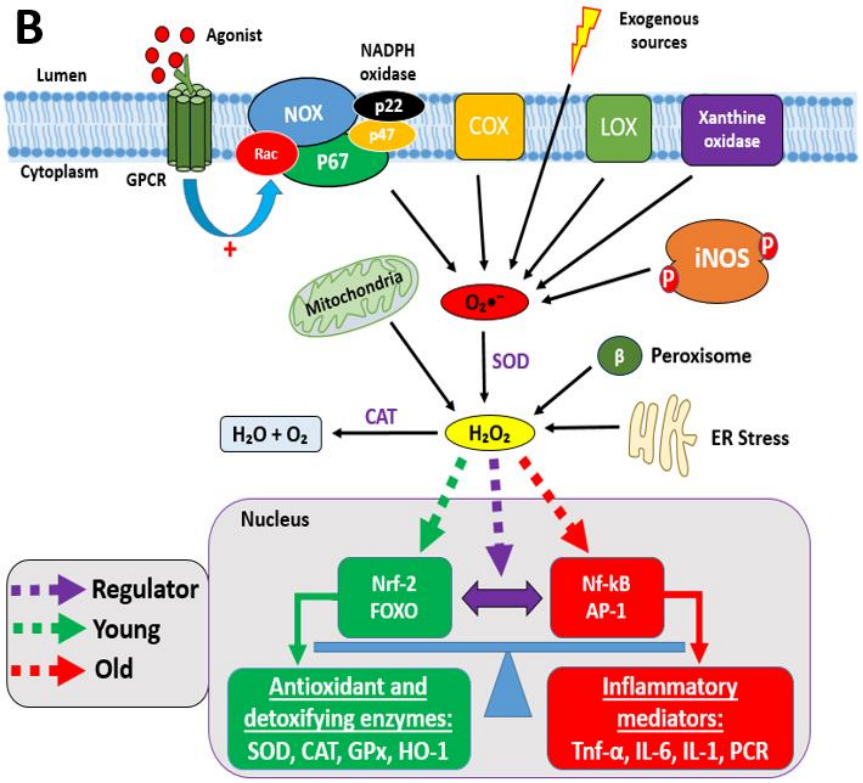

B
Agonist
Lumen
Cytoplasm
GPCR
NADPH oxidase
NOX
p22
p47
Rac
P67
Exogenous sources
COX
LOX
Xanthine oxidase
iNOS
P
Mitochondria
O2•-
SOD
Peroxisome
CAT
H2O + O2
H2O2
ER Stress
Nucleus
Nrf-2 FOXO
Nf-kB AP-1
Antioxidant and detoxifying enzymes: SOD, CAT, GPx, HO-1
Inflammatory mediators: Tnf-α, IL-6, IL-1, PCR
Regulator
Young
Old

Figure 5

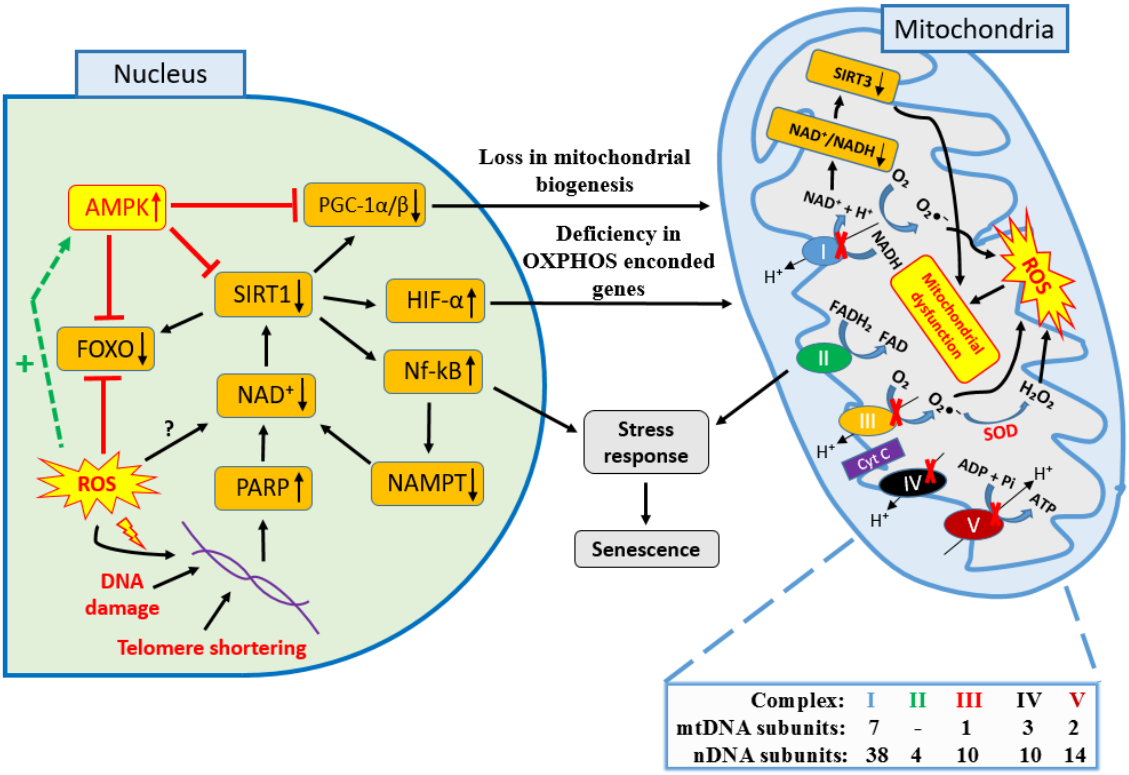

Nucleus
Mitochondria
AMPK
PGC-1α/β
SIRT1
HIF-α
FOXO
Nf-kB
NAD+
PARP
NAMPT
ROS
DNA damage
Telomere shortering
Loss in mitochondrial biogenesis
Deficiency in OXPHOS enconded genes
Stress response
Senescence
SIRT3
NAD+/NADH
Mitochondrial dysfunction
SOD
Cyt C
ADP + Pi
ATP
Complex: I II III IV V
mtDNA subunits: 7 - 1 3 2
nDNA subunits: 38 4 10 10 14

Figure 6

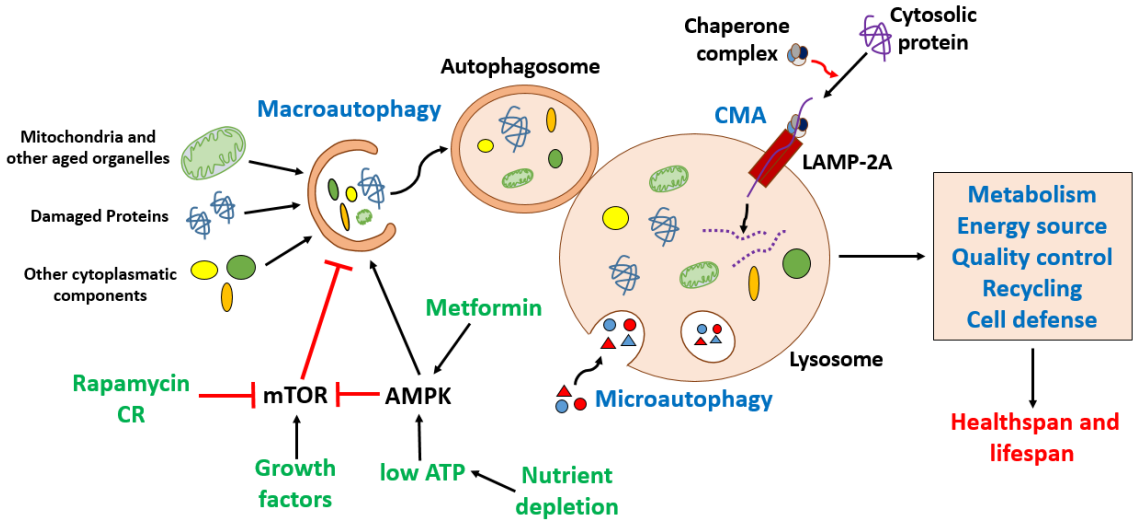

Chaperone complex
Cytosolic protein
Autophagosome
Macroautophagy
CMA
LAMP-2A
Mitochondria and other aged organelles
Damaged Proteins
Other cytoplasmatic components
Metabolism
Energy source
Quality control
Recycling
Cell defense
Metformin
Lysosome
Rapamycin
CR
mTOR
AMPK
Microautophagy
Healthspan and lifespan
Growth factors
low ATP
Nutrient depletion

Figure 7

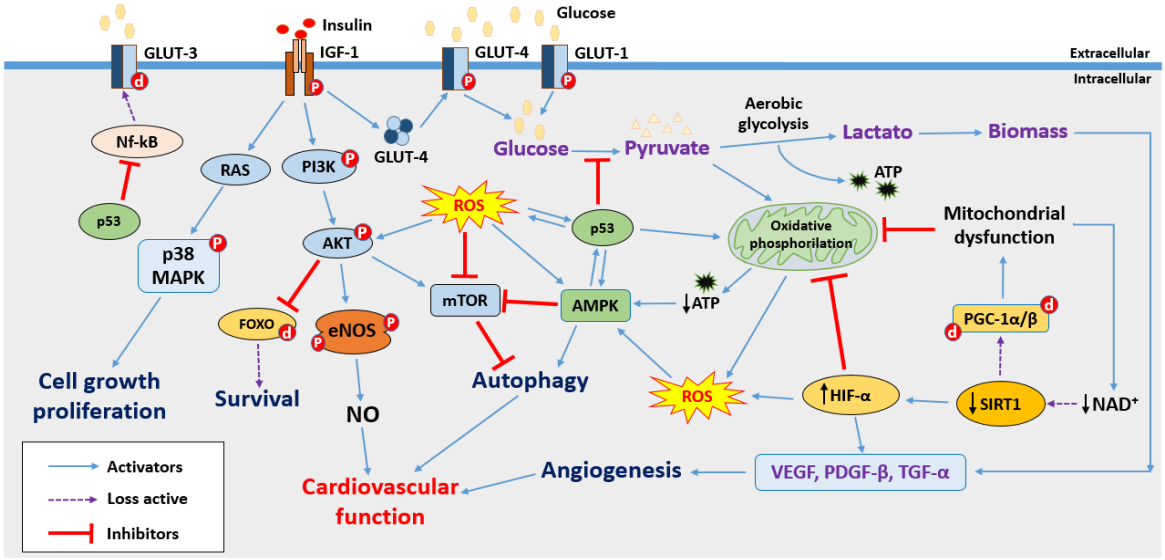
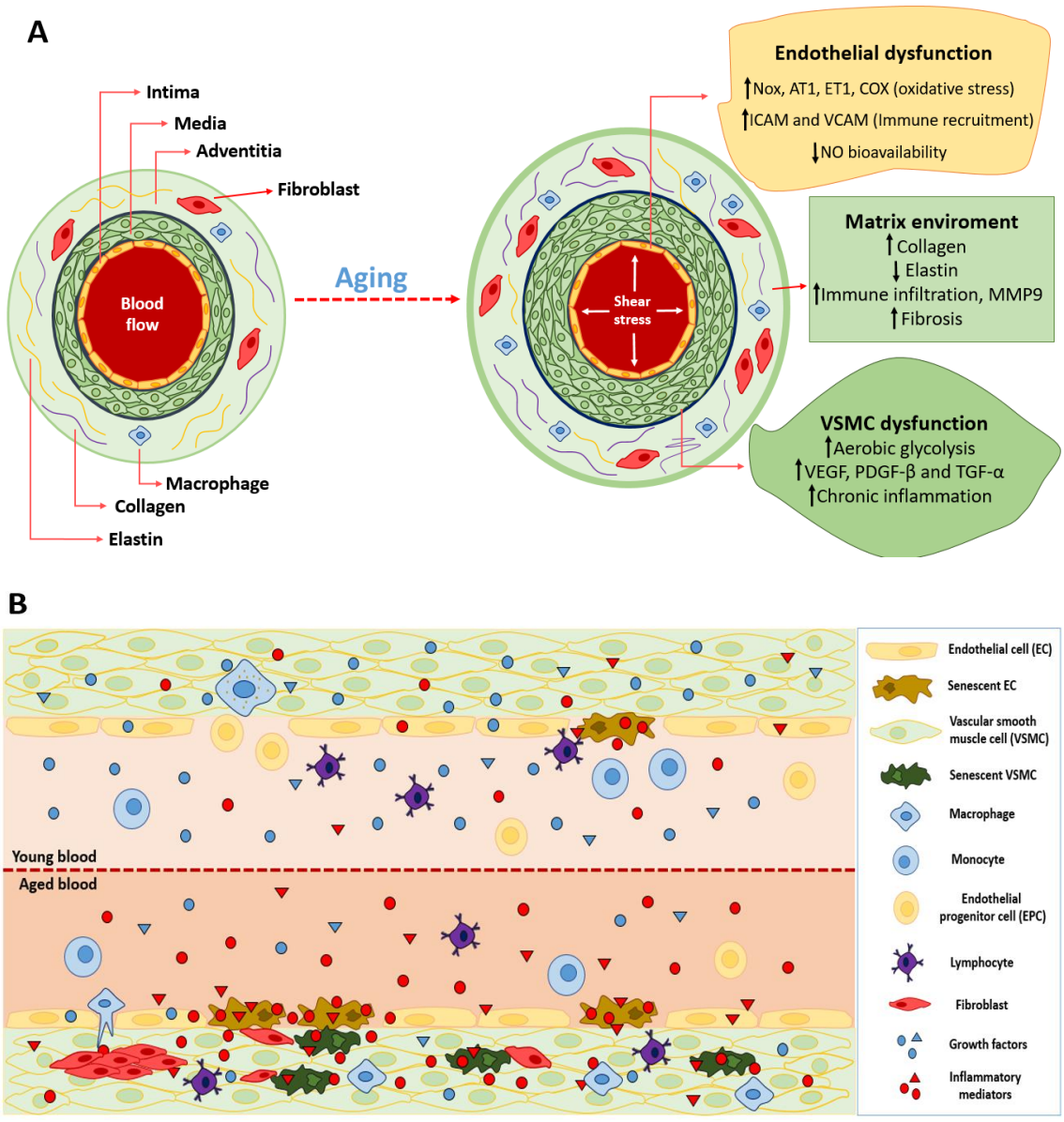

Insulin
Glucose
GLUT-3
IGF-1
GLUT-4
GLUT-1
Extracellular
Intracellular
Nf-kB
RAS
PI3K
GLUT-4
Glucose
Pyruvate
Aerobic glycolysis
Lactato
Biomass
ATP
p53
ROS
p53
Oxidative phosphorilation
Mitochondrial dysfunction
p38 MAPK
AKT
mTOR
AMPK
ATP
FOXO
eNOS
PGC-1α/β
Cell growth proliferation
Survival
NO
Autophagy
ROS
HIF-α
SIRT1
NAD+
Activators
Loss active
Inhibitors
Cardiovascular function
Angiogenesis
VEGF, PDGF-β, TGF-α

Figure 8


A
Intima
Media
Adventitia
Fibroblast
Blood flow
Macrophage
Collagen
Elastin
Aging
Shear stress
Endothelial dysfunction
↑Nox, AT1, ET1, COX (oxidative stress)
↑ICAM and VCAM (Immune recruitment)
↓NO bioavailability
Matrix enviroment
↑Collagen
↓Elastin
↑Immune infiltration, MMP9
↑Fibrosis
VSMC dysfunction
↑Aerobic glycolysis
↑VEGF, PDGF-β and TGF-α
↑Chronic inflammation
B
Young blood
Aged blood
Endothelial cell (EC)
Senescent EC
Vascular smooth muscle cell (VSMC)
Senescent VSMC
Macrophage
Monocyte
Endothelial progenitor cell (EPC)
Lymphocyte
Fibroblast
Growth factors
Inflammatory mediators